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JOURNAL OF TAIWAN AGRICULTURAL RESEARCH
Vol.73 No.4 2024

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ISSN 2790-086X
9 772790 086007

Published by Taiwan Agricultural Research Institute, Ministry of Agriculture

台灣農業研究 JOURNAL OF TAIWAN AGRICULTURAL RESEARCH Vol.73 No.4 2024

ISSN 2790-086X
DOI:10.6156/JTAR

台灣農業研究
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農業部農業試驗所出版

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台灣農業研究

第七十三卷　第四期　　　中華民國一一三年十二月三十一日

出版者　農業部農業試驗所
發行人　林學詩
總編輯　石憲宗
地址　413008 臺中市霧峰區萬豐里中正路 189 號
網址　https://www.tari.gov.tw/jtar
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台灣農業研究 (J. Taiwan Agric. Res.) 73(4):225–233 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0001

專題論述

大花咸豐草的繁殖與競爭策略

林訓仕[1]　李啟陽[2]　楊文欽[3]　何佳勳[4]　蕭巧玲[5,*]

摘要

林訓仕、李啟陽、楊文欽、何佳勳、蕭巧玲。2024。大花咸豐草的繁殖與競爭策略。台灣農業研究 73(4):225–233。

大花咸豐草 (*Bidens pilosa* L. var. *radiata* Sch. Bip.) 為菊科鬼針草屬植物，是臺灣 20 種危害力最高的入侵雜草之一。有幾項特質使之成為優勢入侵種，包括環境適應性廣、種子發芽強勢及降低自交比例等，是大花咸豐草能快速拓展棲地的特性，幫助其於不同環境間落地生存時，迅速適應新棲地與拓殖繁衍範圍。大花咸豐草亦會與它種植物高度競爭環境資源與生存空間，讓共域生長之鄰近植物逐漸消失，再加上根部分泌的酚類化合物對其它植物產生剋他作用 (allelopathy)，能使大花咸豐草在短時間內大量繁殖並占有優勢。本文擬探討大花咸豐草繁殖適應性、競爭機制及剋他物質，藉以瞭解其快速入侵的特性，提供防治管理參考。

關鍵詞：剋他作用、菊科、競爭、入侵植物、繁殖。

前言

入侵植物 (invasive plant) 能快速的占據引入地，多數歸因於較在地植物更優勢的適應性，Bloosey & Nötzold (1995) 提出的『增加競爭力演化假說』(Evolution of Increased Competitive Ability hypothesis; EICA hypothesis)，說明了入侵植物能成功地在引入地取得優勢可能擁有 2 個要件：其一，在相同的生長環境下，入侵植物能較原生植物更具適應性且生產更高的生物量；其二，入侵植物在引入地缺乏專一性天敵 (specialist herbivore)，使植體原本用於抵禦天敵的資源進行再分配，移轉至個體生長繁殖，或用來防禦非專一性天敵 (generalist herbivore)。入侵植物所分泌的各種剋他物質 (allelochemicals)、入侵植物與土壤群落圈的互利共生及對土壤養分的利用效率等 (Deba *et al*. 2007; Cui & He 2009)，亦被認為是入侵植物能與在地植物抗衡的原因。這些研究與假說，都證明了入侵植物具有多樣化的侵占機制，才能在引入地或入侵地中擴散分布 (Cui & He 2009)。

咸豐草被歸類在菊科 (Asteracease) 鬼針草屬 (*Bidens*)，咸豐草在臺灣有 3 個變種，分別是：白花鬼針 (*B. pilosa* L. var. *pilosa*)、小白花鬼針 (*B. pilosa* L. var. *minor* (Blume) Sherff) 及大花咸豐草 (*B. pilosa* L. var. *radiata* Sch. Bip.) (Peng *et al*. 1998; Budumajji & Solomon Raju 2018)。未開花前，三者植株外觀時常被混淆為一體。大花咸豐草為 1 年生至多年生草本植物，莖直立，略帶四菱形，無毛或略披極稀疏柔毛，高 30–100 cm 左右。葉片形態多樣化，成熟葉為單數羽狀複葉，上位葉葉緣鈍齒狀，葉尖鈍形；中位葉葉緣鈍齒狀，葉尖為漸尖形；下位葉葉緣鈍齒狀，葉尖鈍形。花屬頭狀花序，具舌狀，帶白色或略有紫紅色。種子

收件日期：2024 年 6 月 26 日；接受日期：2024 年 8 月 15 日。
[*] 通訊作者：clhsiao@tari.gov.tw
[1] 農業部臺中區農業改良場助理研究員。臺灣 彰化縣。
[2] 農業部農業試驗所應用動物組助理研究員。臺灣 臺中市。
[3] 中央研究院農業生物科技研究中心研究員。臺灣 臺北市。
[4] 農業部農業試驗所作物組助理研究員。臺灣 臺中市。
[5] 農業部農業試驗所作物組副研究員。臺灣 臺中市。

為細長的牙狀瘦果，並有淡黃色瘤基向上的短刺，頂端有下彎或鉤狀芒刺 2 或 3 根，芒刺則著生 2 列小倒刺 (Peng *et al*. 1998; Hsu & Lin 2005; Bartolome *et al*. 2013; Xuan & Khanh 2016; Hsueh 2021)。大花咸豐草已逐漸取代白花鬼針與小白花鬼針的棲地，外表形態與後二者略趨相似，不過仍可在花與種子的細微構造上稍作區分。大花咸豐草的舌狀花較白花鬼針與小白花鬼針大，至於種子則能以瘦果的芒刺數目、倒刺的列數及數目作為鑑別 (Wang & Zhang 2002; Hsu & Lin 2005)。

咸豐草原產於南美洲，廣泛分布在溫帶與熱帶地區 (Bartolome *et al*. 2013)，也傳播散布於亞洲熱帶與亞熱帶地區。臺灣於 1976 年由琉球引進大花咸豐草作為蜜源植物，至此之後便落地生根，直至 1984 年才首次報導為臺灣的新紀錄植物 (Hsu & Lin 2005; Bartolome *et al*. 2013)，主要分布於臺灣中低海拔地區，包括廢耕園區、休耕地及道路兩旁等，它與其他植物高度競爭環境資源的能力，可能是在臺灣持續分布的原因 (Hsueh *et al*. 2020a)。

臺灣約有 130 種非本土雜草入侵果園、農田或非耕地 (Chiang 2005)，而大花咸豐草則是名列於 20 種危害最嚴重的入侵植物之一 (Hsu & Kao 2014)，已逐漸取代原生種白花鬼針與小白花鬼針 (Chien *et al*. 2009)，本文擬就大花咸豐草繁殖能力與其特有的競爭機制，瞭解其入侵與生長特性，並探討其剋他作用對鄰近植物造成的影響，以作為後續防除與管理之參考。

大花咸豐草繁殖策略

美國農業部自然資源保育局 (Natural Resources Conservation Service; NRCS) 所公布的大花咸豐草資料為 1 年生的生活史，但入侵臺灣多年後發現大花咸豐草生活史已逐漸轉為 2 年生，甚至可達多年生 (Bartolome *et al*. 2013)。Müller-Schärer *et al*. (2004) 認為入侵植物在入侵後的生活史或生長策略發生改變的原因可能是少了捕食天敵的威脅，才演變為多年生，藉以提高繁殖率。由此可知，大花咸豐草在入侵後能隨環境變異而調整自身生長與適應性，拓展族群繁衍範圍。本節探討大花咸豐草之環境適應與遺傳特性，以瞭解其與生俱來的優勢如何與鄰近植物爭奪生存空間。

生殖策略

大花咸豐草能成功入侵的機制，除了適應性廣之外，其花部生物學特性也是協助族群拓殖的機制之一。Huang & Kao (2014) 研究大花咸豐草、小白花鬼針及白花鬼針三者之花部生物學，以瞭解何以大花咸豐草族群能較後兩者為多。將三者分別以套袋與開放授粉處理，經 3–4 wk 後收集頭狀花序，以套袋處理的大花咸豐草，單一頭狀花約有 41 個可孕的管狀花，在開放授粉的環境，其管狀花數量顯著高於套袋處理；比較 3 個變種間管狀花的數量，顯示大花咸豐草少於其他兩者。續檢視套袋與開放授粉之結實率，發現套袋處理之大花咸豐草幾乎無法結實，但於開放授粉處理之結實率卻有 57.7%，因此大花咸豐草可能為自交不親和性。雖然 3 個變種皆為異交植物，但其餘兩者在套袋中仍可自交結實，似乎為自交親和 (Huang & Kao 2014)。

花粉/胚珠比 (pollen/ovule ratio; P/O ration) 常被植物學家應用於植物繁育系統的研究上，此可反映植物在授粉過程中使子房胚株受精後之最大結實量。換言之，越有效的授粉模式，其 P/O 比越低，自花授粉 (autogamous) 植物的 P/O 比常比異花授粉 (xemogamous) 低，當 P/O 比高於 5,800 以上時，可視為自交不親和 (Mione & Anderson 1992; Huang & Kao 2014)。P/O 比並非是一項判斷自花或異花授粉的絕對標準，其仍受環境因素與植物特性差異而變化，惟在控制環境下，可作為辨識的方式之一 (Mione & Anderson 1992)。據此以分析三者之 P/O 比，大花咸豐草、小白花鬼針及白花鬼針分別為 8,189、2,053 及 1,613，顯示 3 個變種間有不同的繁殖特性，大花咸豐草具有高度的自交不親和性以防止自交弱勢，且個體間具有顯著的異質性，為異交植物。此特性可幫助大花咸豐草於不同環境間生存，在拓殖新棲地時能調整其生長方式，以因應各種逆境 (Huang & Kao 2014)。

三種鬼針草屬的植物都屬異交，須由授粉

昆蟲協助完成其異花授粉的工作，惟大花咸豐草具有強烈的自交不親和性 (Huang & Kao 2014; Budumajji & Solomon Raju 2018)。Huang & Kao (2014) 發現次級花粉呈現機制 (secondary pollen presentation) 可能是大花咸豐草避免自交的方式。此機制是指花粉從花藥中散出後會落到非花藥的花部結構上 (如雌蕊、花被、花冠或雄蕊非花藥部分)，經由授粉者 (通常為昆蟲) 於此結構上沾黏並傳至異花柱頭上，可避免花粉-雌蕊間的干擾 (pollen-pistil interference)，此項特性常見於菊科、桔梗科及豆科等。由於次級花粉呈現機制是由於雌雄蕊異時成熟 (dichogamous) 的關係，根據花藥筒與花柱的外觀形態差異，可將 3 個變種的次級花粉呈現機制分為 6 個階段 (stage A–F)。分析 6 個階段的各變種間的花粉活性，發現三者從 stage A 至 stage F 的花粉活性會逐漸下降，雖然 3 個變種間並無顯著差異，但由於小白花鬼針與白花鬼針自交後能夠結實，大花咸豐草卻無法產生瘦果，顯示次級花粉呈現是協助大花咸豐草自交不親和性的機制。利用雌雄蕊異時成熟達到免除花粉-柱頭間不親和的交互作用，來減少自交比例，促進異花授粉，以產出具有優勢的後代適應更多不同的環境 (Huang & Kao 2014; Budumajji & Solomon Raju 2018)。

種子形成時間的調整

由於大花咸豐草生長旺盛，原為單軸生長的植株在受到外力干擾能於 10 d 左右從葉腋再生出側枝，並快速開花傳播瘦果。瘦果傳播後則會逐漸萎凋，新的側枝又會重新長出，如此周而復始地繁衍，以占據引入地 (Huang 2008; Shimamoto *et al*. 2011; Hsu & Kao 2014)。

Shimamoto *et al*. (2011) 發現人為的修剪會刺激大花咸豐草產生超補償效應 (overcompensation)，提高無性繁殖機會。其在種植 32 wk 期間，以每 4、8、12、16 wk 修剪頻率及不修剪等 5 個處理，探討對大花咸豐草開花結實的差異。試驗發現，以 12 wk 與 16 wk 的間隔修剪 1 次會刺激大花咸豐草長出側枝並開花結實，展現出超補償效應，亦即經過此頻率修剪後，其植株再生繁殖力超過不修剪處理。每 4 wk 或 8 wk 修剪的頻率則能顯著抑制大花咸豐草開花與結實，尤其是修剪頻率縮短至 4 wk，種子結實率僅有 2.7%。若為了控制大花咸豐草瘦果傳播，以少於 8 wk 的頻率修剪 1 次，能顯著地防止其繁衍範圍擴大。

種子生態與傳播

環境條件是影響大花咸豐草種子萌芽的重要因素，包括水分、溫度、光照、覆土深度及鹽分逆境等，在最適的條件下有助提高種子發芽與出土 (Reddy & Singh 1992; Ramirez *et al*. 2012; Hsu & Kao 2014)。大花咸豐草由種子 (瘦果) 繁殖，單株的種子量約有 3,000–6,000 粒，且瘦果儲存 3–5 年仍具有 80% 發芽率 (Reddy & Singh 1992; Xuan & Khanh 2016)，可見其繁衍力足以影響他種植物生存。

大花咸豐草的瘦果為黑色長條狀，表面粗糙具有 4 條稜線，長度約 8–12 mm，寬度約 0.8 mm，頂端披有淡黃色瘤基向上短芒刺，約 2–4 條，每個芒刺著生 2 列小倒刺，每列約有 5–7 枚倒刺 (Hsu & Lin 2005; Chauhan *et al*. 2019)。瘦果芒刺上方的倒刺有利於勾住人類、動物的毛髮或衣物，隨著移動至他處進行傳播或擴散 (Chauhan *et al*. 2019)。此外，瘦果著生位置也影響大花咸豐草傳播的能力，研究發現著生於果序中央位置的瘦果比外圍的瘦果長，也相對較重，長度分別為 94.4 mm 與 71.8 mm，重量則為 21 g 與 1.73 g。果序中央位置的新鮮瘦果發芽率為 88%，而外圍瘦果則為 52%，顯示中央瘦果比外圍瘦果更容易附著於潛在的物體上去進行有效傳播 (Rocha 1996)。

瘦果的長度與傳播遠近也有相關，大花咸豐草細小的瘦果除了容易受動物或人力遷徙而傳播外，也能靠著風力與水來傳播 (Budumajji & Solomon Raju 2018; Chauhan *et al*. 2019)。較長的瘦果表面疣狀黃色瘤基較少，整體重量較輕，容易受到風力散播到離親代地點較遠的地方，並在各種條件下容易發芽；短瘦果相對於長瘦果有較多疣狀黃色基瘤，並相對較重，常散落在親代附近，且於類似棲息地的特定發芽條件下生長 (Budumajji & Solomon Raju 2018)。大花咸豐草種子以多種傳播方式來確

保後代的繁衍，顯見其族群的優勢能逐漸於各種環境下擴張。

種子發芽的環境因子

大花咸豐草種子對水分需求較為敏感，種子發芽率隨著滲透壓增加而顯著降低，滲透壓約在 -0.4 MPa 以上時，發芽率會減少至 50% 以下，當達 -0.75 MPa 的發芽率則僅剩 3%。胚根長度同樣受到滲透壓增加的影響而抑制，-0.75 MPa 的胚根長度只有 0.3 cm，與正常胚根 7.3 cm 差距甚大 (Reddy & Singh 1992)。以 6 種不同 NaCl 濃度測試大花咸豐草種子發芽情形，顯示發芽率與胚根長度隨著濃度增加而下降，在 100 mM 下發芽率低於 13%，在 150 mM 仍有少數種子萌發，顯示大花咸豐草於 10–150 mM 鹽分環境下可生長 (Ramirez *et al*. 2012)。

對於土壤酸鹼度忍受性廣也是大花咸豐草對環境高度順應的能力之一 (Hsueh & Chang 2017)。在光照情形下，大花咸豐草即使在 pH 2.5–7 下其發芽率仍達 50–90%，在黑暗下，土壤酸鹼度達到 pH 11 甚至仍有 80% 的發芽率，顯見大花咸豐草對土壤酸鹼度的耐受性廣，有利分布於不同地區，拓殖族群密度與領域 (Hsueh & Chang 2017)。

由白花鬼針、小白花鬼針及大花咸豐草發芽溫度的表現可知，三種咸豐草的最適發芽溫度為 16–28℃，但超過 28℃與30℃時，發芽率將呈現下降趨勢。以 16℃檢視達 50% 發芽率所需的天數，以小白花鬼針最快，平均只要 4 d 即可達到，大花咸豐需 5–6 d 為次之，白花鬼針則需 6–7 d 才能到達 50% 發芽率 (Hsu & Lin 2005)。在 24℃下，照光有助提高小白花鬼針鬼針草發芽率達 97%，大花咸豐草則為 91.5%，白花鬼針之 43.5% 發芽率最低。黑暗下小白花鬼針的發芽率迅速下降至 14.5%，而大花咸豐草則影響較輕微，發芽率仍有 79%，表示大花咸豐草的發芽對光照需求並不明顯 (Hsu & Lin 2005)。Hsueh *et al*. (2020b) 推測大花咸豐草發芽隨環境影響而異，培養皿黑暗處理之發芽率高於光照處理，但在有介質的盆栽中明顯的有光照需求，顯示光照雖然不是大花咸豐草主要的發芽先決條件，但可能是一項刺激因子 (Chauhan *et al*. 2019)。

再以溫度輔助光照來檢視可能影響發芽的關鍵。由於 *B. alba* 與大花咸豐草類群適應性相似，因此以 *B. alba* 在不同溫度/光照對發芽率的影響來說明大花咸豐草可能的溫度與光度交感之適應性。試驗結果發現，*B. alba* 的萌芽明顯受溫度與光照影響，在較低溫的環境 (day/night (D/N): 15/10℃與 20/15℃)，黑暗狀態有助發芽 (Ramirez *et al*. 2012)。

由覆土深度的試驗中可知，大花咸豐草比其它兩種鬼針有較佳之發芽率，且較不會因 0–5 cm 的覆土而影響發芽率，然而發芽率最高者為分布於土壤表面，發芽率隨著土壤深度增加而呈線性下降。小白花鬼針則對覆土深度最為敏感，大花咸豐草在土壤深度達 8 cm 時的發芽率仍有 10%，但 10 cm 以上者幾乎無種子發芽 (Reddy & Singh 1992; Hsu & Lin 2005)。從大花咸豐草的旺盛發芽率可以說明，為何其進入臺灣後能快速取代原有白花鬼針與小白花鬼針的棲地 (Hsu & Lin 2005)。此外，不整地或低整地的農耕操作下，有助增加大花咸豐草的發芽率 (Reddy & Singh 1992)，在常出現大花咸豐草的田區中，透過耕犁整地或許能減少族群數增加。

溫度對於種子萌發相當重要 (Reddy & Singh 1992; Ramirez *et al*. 2012)，由 2 種溫度檢視鬼針與大花咸豐草發芽情形的差異，在較高溫的 28℃環境下，對鬼針與大花咸豐草的發芽率無明顯差異，鬼針之發芽率甚至稍快於大花咸豐草；但在 18℃時，鬼針之發芽明顯受到低溫抑制，且發芽率顯著地低於大花咸豐草，顯示大花咸豐草在高溫環境下可與鬼針共域競爭，在低溫季節能侵入鬼針不利生存的空間，發展其族群範圍 (Hsu & Kao 2014)。

營養繁殖能力

為了瞭解鬼針與大花咸豐草的無性繁殖能力，將 4 mo 大的植株剪除第 7–15 節中的 1–2 個節點，並切除對生葉中的一部分葉面積，檢視兩者側枝生長情形。試驗發現在修剪後的第 10 天，大花咸豐草已有不定根長出，約 20 d 後的不定根長度已超過 75 cm，反觀鬼針之不定根幾乎不生長。再觀察側枝生長情形，大花咸豐草自修剪第 10 天後已有側枝長出，20 d

後的側枝約有 6 cm 以上。由此可知，大花咸豐草不定根與側枝生長能力較鬼針強，其營養繁殖能力亦較鬼針高出許多。這項特徵有助大花咸豐草進行無性繁殖，在側枝接觸土壤後能快速長出不定根，形成新的個體，並且也能藉由側枝獲得較多的生長空間，有利拓展族群面積，以抑制其他植物生存面積 (Shimamoto *et al*. 2011; Hsu & Kao 2014)。

生長的競爭與剋他作用

入侵植物侵擾的衝擊不僅影響跨域生物多樣性的平衡，對農作物的威脅與防治成本的經濟支出等，已成為全球性的研究議題。為何能成功入侵，除了對環境適應性廣的特徵外，與其它物種間競爭養分、水分及光線的能力亦是關鍵 (Deba *et al*. 2007; Cui & He 2009; Rashid *et al*. 2010)，藉由競爭作用提高生長速率等機制來妨礙其它物種獲取相同環境資源。剋他物質是另一項控制地域範圍的策略，利用葉片、莖部或根部分泌的多種植化素以抑制它種發芽或生長 (Bais *et al*. 2003; Deba *et al*. 2007; Hsu & Kao 2009; Hsu & Kao 2014)，這些特性使入侵植物能成為一地的優勢者。

生長的競爭

鬼針草屬在臺灣除了前述提及的白花鬼針、小白花鬼針及大花咸豐草 3 個變種外，尚有鬼針 (*B. bipinnata* L.)、鬼針舅 (*B. biternata* (Lour.) Merr & Sherff) 及狼把草 (*B. tripartite* L.) 等，其中除了大花咸豐草與鬼針為外來植物，其餘為原生種 (Peng *et al*. 1998; Budumajji & Solomon Raju 2018)。鬼針與大花咸豐草在臺灣都屬入侵種植物，但現今的鬼針族群已逐漸減少，由於兩者生長習性相同，在多數地方共域生長，利用的環境資源高度重疊，歸咎其原因可能為大花咸豐草比鬼針能獲得較大生物量有關 (Hsu & Kao 2014)。

分析大花咸豐草與鬼針的生長差異，鬼針開花時間較早，因此在後期養分大多分配至生殖生長，與大花咸豐草同時競爭。大花咸豐草累積生物量的能力高於鬼針 1 倍以上，在鬼針進入生殖生長後期，正值大花咸豐草甫接近生殖生長。因此，大花咸豐草有較高的根重比，尚包括側枝重、根/莖比亦較鬼針優勢，能獲取較多的養分，使植株將養分充分貢獻於生長。由此顯示，鬼針的繁殖策略傾向快速完成生活史與產生種子，而大花咸豐草營養生長期比鬼針長，植株較為高大，兩者共域發展時，鬼針可能會受大花咸豐草植株遮蔭而影響生長 (Hsu & Kao 2014)。

相鄰植物在養分缺乏的條件下，兩者間的養分競爭會更顯得強烈，因為植物根部分泌的物質會互相減少或抑制對方礦物質吸收的程度，來提高自身養分的溶解性、可利用性及使用效率 (Jabran *et al*. 2013; Scavo *et al*. 2019)。大花咸豐草在與香附子 (*Cyperus rotundus* L.) 的生長競爭試驗中，將兩者同時種植於盆栽，以觀察植株地上部與根部的競爭關係，經過 10 wk 的共域生長結果發現，大花咸豐草會將養分優先集中在地上部，而香附子則是將養分貢獻在根系的發展中 (Hsueh *et al*. 2020a)。再利用活性碳吸附大花咸豐草與香附子的根分泌物，比較兩者間競爭資源的表現，以根部乾重來說，添加活性炭後，大花咸豐草的根重明顯減少 50%，而香附子根重則略有增加，顯示活性炭吸附了大花咸豐草根部分泌物後，對香附子的競爭力減少，而促進香附子根重增加 (Hsueh *et al*. 2020a)。

剋他作用

酚類化合物是多數入侵植物的主要剋他物質成分，這些剋他物質能夠抵禦引入地之病原菌或食草動物的威脅，亦能抑制其他植物生長，以成功占據引入地 (Deba *et al*. 2007; Hsu & Kao 2009; Rashid *et al*. 2010; Hsueh *et al*. 2020a)。

利用大花咸豐草根、莖及葉的水萃液處理與其共域生長之鬼針與白花霍香薊 (*Ageraturem conyzoides*)，試驗顯示大花咸豐草之根部萃取液顯著影響自身與鬼針之發芽率，鬼針受抑制的情形高於大花咸豐草，表示鬼針與大花咸豐草同時生長時易被阻礙。白花霍香薊的發芽率對於大花咸豐草不同部位水萃取液皆無明顯影響，證明當白花霍香薊與大花咸豐草共域時，兩者可相互競爭生長 (Hsu & Kao 2009)。

將大花咸豐草植株分為葉、莖及根 3 部分，利用不同濃度水萃液測試稗草 (*Echinochloa crus-galli*) 與蘿蔔 (*Raphanus sativus*) 對發芽、胚軸及胚根伸長的抑制情形。大花咸豐草各部位的水萃液能抑制稗草發芽，惟莖部與葉部不同濃度間無顯著差異，稗草胚軸與胚根受抑制的現象隨著水萃液的濃度增加而增加。大花咸豐草水萃液對蘿蔔的抑制與稗草類似，葉部、莖部及根部對蘿蔔萌芽皆有 83.3% 的抑制率，但不同濃度間無顯著差異，大花咸豐草對蘿蔔胚軸與胚根抑制生長的情形同樣受到水萃液濃度增加而增加。此外，稗草受到抑制的現象高於蘿蔔，顯示此類的剋他物質似乎可做為天然的除草劑 (Deba *et al*. 2007; Rashid *et al*. 2010)。

續分析大花咸豐草剋他物質組成分，發現最主要的酚類化合物共有 6 種，以咖啡酸 (caffeic acid) 含量最多，普遍存在於葉、莖及根部，其次為焦兒茶酚 (pyrocatechin) 與阿魏酸 (ferulic acid) 等，蘿蔔與稗草受到剋他作用的影響可能是由上述物質引起 (Deba *et al*. 2007)。咖啡酸是常見的剋他物質，對於他種植物之種子萌芽與胚軸生長有顯著抑制作用，尤其是苗株發根期對咖啡酸的刺激更為敏感 (Batish *et al*. 2008)。分析咖啡酸對 3 個發根階段 (rhizogenesis) 之綠豆苗酵素活性的變化，其發根階段分別為根原基出現期 (root initiation; RI，發芽後 3 d)、根露出期 (root expression; RE，發芽後 5 d) 及根系表現期 (post-expression; PE，發芽後 7 d)。不論是蛋白酶 (proteases)、過氧化酶 (peroxidases; PODs) 及多酚氧化酶 (polyphenol oxidases; PPOs)，皆顯示出三者活性隨著咖啡酸濃度與發根天數增加而增加，而酚類化合物含量卻呈現相反趨勢。3 種酵素活性與發根階段之木質化與酚類化合物代謝相關，因此當咖啡酸促進上述酵素活性增加表示會刺激後續蛋白質水解與木質化來因應咖啡酸的逆境，但在高濃度下 (1,000 μM) 的酵素活性就明顯受到抑制，也使得酚類化合物含量累積 (Batish *et al*. 2008)。

大花咸豐草剋他物質次高的焦兒茶酚為兒茶酚類之一，常見於具剋他作物之植物抑制鄰近植物生長之物質。斑點矢車菊屬 (*Centaurea maculosa*) 是南美洲的外來植物 (Bais *et al*. 2003)，經由萃取分餾發現根部所分泌的兒茶酚是其影響原生種植物的剋他物質，當兒茶酚 (100 μg mL^{-1}) 添加到矢車菊屬 (*C. diffusa*) 與阿拉伯芥 (*Arabidopsis thaliana*) 上，發現這二個植物的根尖分生組織會比延長區更早發生細胞質的縮合 (condensation)，縮合反應會從分生組織往後移到中心柱而引起組織滲漏死亡。為了更加了解這樣的改變，將二植物的根部加入活標本用的螢光染劑-二乙酸螢光素 (fluorescein diacetate; FDA)，再加入 100 μg mL^{-1} 兒茶酚，10 min 後發現，分生組織與中心柱細胞會最先受到影響，根的活性逐漸減少，且 FDA 會漸漸不呈現；在施用 55 min 後，更使細胞死亡。兒茶酚在根毛上也會影響細胞質的流動，並使細胞質往根尖滲漏。

Bais *et al*. (2003) 也發現兒茶酚會引起活化氧族 (reactive oxygen species; ROS) 的產生。利用對 ROS 有感受性的螢光染劑-二氯螢光黃 (dichlorofluorescein; DCF) 呈像。當阿拉伯芥與矢車菊屬被兒茶酚誘導產生大量 ROS 時，螢光反應會很快 (10 s 內) 呈現出來，起先會在根尖組織隨後會往後移至延長區細胞內，這樣移動模式類似由兒茶酚引起的細胞死亡模式，從 DCF 的呈像中可得知，在根毛上 ROS 會更快速的產生。因此，兒茶酚會引起根細胞的死亡，藉此抑制其他植物生長，與斑點矢車菊同為菊科之大花咸豐草所分泌的焦兒茶酚可能也是以此機制抑制它種植物生長。

結語

大花咸豐草在臺灣有紀錄以來已超過 30 年，廣泛存在於農田與非耕地，因瘦果數量多且具倒鉤刺，常增加其防治及管理之困擾。由於其具有強勢的環境適應能力與剋他作用，並兼具無性繁殖與異交有性繁殖的系統，加上臺灣適宜的氣候，才能使得大花咸豐草擴散，並威脅其他植物的生存。如欲降低大花咸豐草族群密度，可從繁殖特性著手，最主要須減少瘦果的數量，在未大量開花結種子前，吾人可利用適當修剪頻率或在未開花前即拔除，來降低土壤種子庫數量，以控制傳播範圍。此外，大

花咸豐草所分泌的剋他物質，鄰近植物幼苗階段最易受到影響。因此，作物幼苗期如有零星出現大花咸豐草，應須儘速拔除，以減少幼苗根系受損的可能。經修剪或刈除之殘株也須儘速移出農田中，因為大花咸豐草之不定根與側枝生長能力強，即便是小於 3 cm 的枝條，只要帶有芽點或不定根生長點，就有可能以無性繁殖形成新植株。

大花咸豐草的發芽率隨著土壤深度增加而呈線性下降，當深度達 8 cm 以上時，發芽率降至 10% 以下，在有大花咸豐草優勢存在的農田，於下期作物開始種植或播種前，可經過翻耕，將表層雜草種子埋入較深層土壤中，來減少表層的種子可能的萌芽數量。此外，透過翻耕後，可再於田區保持 5 cm 以上的水位達 3–4 d，亦有減少萌芽的機會，藉以降低大花咸豐草種子密度，達到防除與管理的功效。

本文透過文獻搜尋瞭解大花咸豐草的繁殖策略、競爭機制及剋他作用外，亦發現大花咸豐草在抑制他種植物的競爭策略中，採用剋他物質對抗與之競爭的植物，其剋他物質主要為酚類化合物，能對鄰近的植物個體與環境產生影響，此類物質同時也具有生物藥理作用 (Bartolome *et al*. 2013; Xuan & Khanh 2016; Liang *et al*. 2020)，在許多國家被當作可食用的傳統食材或民俗醫學用途的植物，來作為藥用植物治療人類或動物的疾病 (Bartolome *et al*. 2013; Liang *et al*. 2020)。未來將接續探討大花咸豐草機能性功能或潛在利用性，以作為相關研究之參考。

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Propagation and Competition Strategies of *Bidens pilosa* L. var. *radiata*

Hsun-Shih Lin[1], Chi-Yang Lee[2], Wen-Chin Yang[3], Chia-Hsun Ho[4], and Chiao-Ling Hsiao[5,*]

Abstract

Lin, H. S., C. Y. Lee, W. C. Yang, C. H. Ho, and C. L. Hsiao. 2024. Propagation and competition strategies of *Bidens pilosa* L. var. *radiata*. J. Taiwan Agric. Res. 73(4):225–233.

Bidens pilosa L. var. *radiata* Sch. Bip., a member of the Asteraceae family, is one of the top 20 most invasive weeds in Taiwan. It possesses several traits that make it a dominant invasive species, including broad environmental adaptability, strong seed germination, and reduced self-pollination rate. These characteristics enable the *B. pilosa* to rapidly expand its habitat, allowing it to quickly adapt and proliferate in new environments. *B. pilosa* also competes intensely with other plants for environmental resources and living space, causing neighboring plants to gradually disappear. Additionally, the phenolic compounds secreted by its roots have allelopathic effects on other plants, enabling the *B. pilosa* to reproduce rapidly and establish dominance in a short period of time. This mini-review aims to explore the reproductive adaptability, competitive mechanisms, and allelopathic substances of the *B. pilosa* to further understand its rapid invasion, as well as to provide a reference for its management.

Key words: Allelopathy, Asteracesa, Competition, Invasive plant, Propagation.

Received: June 26, 2024; Accepted: August 15, 2024.
[*] Corresponding author, e-mail: clhsiao@tari.gov.tw
[1] Assistant Research Fellow, Crop Improvement Section, Taichung District Agricultural Research and Extension Station, Changhua County, Taiwan, ROC.
[2] Assistant Research Fellow, Division of Applied Zoology, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[3] Research Fellow, Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan, ROC.
[4] Assistant Research Fellow, Division of Crop Science, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[5] Associate Research Fellow, Division of Crop Science, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 73(4):235–250 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0002

Research Article

Reutilization of Different Recycled Agricultural Wastes to Culture *Pleurotus eryngii* and Comparison of Their Ingredients

Wei-Sung Li[1], Min-Chien Cheng[2], Wen-Huang Peng[3,*], and Jen-Chieh Tsai[4,*]

Abstract

Li, W. S., M. C. Cheng, W. H. Peng, and J. C. Tsai. 2024. Reutilization of different recycled agricultural wastes to culture *Pleurotus eryngii* and comparison of their ingredients. J. Taiwan Agric. Res. 73(4):235–250.

In this study, bamboo crumb, paddy straw crumb, sugarcane crumb, spent *Pleurotus eryngii* substrate, or spent *Flammulina velutipes* substrate was used to completely or partially replace fresh sawdust to cultivate *P. eryngii*. The nutrients and functional components were compared. The fingerprints and the contents of ergosterol and ergothioneine analyzed using high-performance liquid chromatography (HPLC) were compared. The results revealed that the samples cultivated using spent *P. eryngii* substrate or spent *F. velutipes* substrate had higher crude protein content. The water-soluble extract contents of *P. eryngii* cultured with spent *P. eryngii* substrate were higher than that cultured with sawdust. The total polysaccharide content of *P. eryngii* cultured with sugarcane substrate was significantly higher than that of *P. eryngii* cultured with sawdust, while the total polysaccharide content of *P. eryngii* cultured with bamboo was lower. The high-performance liquid chromatography fingerprint findings revealed the compositions of *P. eryngii* cultured from any agricultural waste substrate were the same. The ergosterol content was between 0.015 and 0.067 mg g^{-1}, and the ergothioneine content was between 0.890 and 2.542 mg g^{-1}. These results indicated that using recycled materials instead of sawdust to cultivate *P. eryngii* to reduce agricultural waste could be a safe and worthwhile solution.

Keywords: *Pleurotus eryngii*, Agricultural waste, Nutrient, Ergosterol, Ergothioneine.

INTRODUCTION

In recent years, because of the increasing awareness regarding environmental protection, global warming, and water and air pollution, people have gradually discovered that the past lifestyle of mass production and consumption has resulted in the production of large amounts of waste that have exceeded the environmental load (Barshteyn & Krupodorova 2016). Taiwan's agriculture is considerably developed, and it generates a large amount of agricultural waste. In the past, farmers mostly used incineration or composting to manage this waste; these techniques adversely affect air quality and the quality of life of people residing in neighboring areas. Taiwan's mushroom cultivation bring us at least 300,000 metric tons per year of spent sawdust waste (spent mushroom substrate) (Wu *et al*. 2020), the non-governmental organization

Received: August 8, 2024; Accepted: October 6, 2024.
* Corresponding author: jenchieh@mail.cmu.edu.tw; whpeng@mail.cmu.edu.tw
1 Associate Research Fellow, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC
2 Associate Research Fellow, Southern Region Branch, Taiwan Livestock Research Institute, Pingtung County, Taiwan, ROC
3 Professor, Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung City, Taiwan, ROC
4 Associate Professor, School of Pharmacy, China Medical University, Taichung City, Taiwan, ROC

(Citizen of the Earth, Taiwan) claims that results in the equivalent of 88 Daan Forest Parks must be cut down, which is harmful to forest environments.

Most agricultural waste is rich in lignocellulosic compounds, the disposal of which is often a problem because they do not decompose easily. In the past, agricultural waste was often treated through combustion (Udayasimha & Vijayalakshimi 2012). However, recent studies have found that agricultural waste can be effectively used for mushroom cultivation, thus benefits the economy and reduces environmental pollution (Kamthan & Tiwari 2017). If rice straw, bagasse, or wastes produced after cultivating edible mushrooms can be recycled to cultivate mushrooms, the deforestation and pollution caused by the disposal of agricultural waste can be reduced, and the mushroom industry can become a completely sustainable and environmentally friendly industry that decomposes agricultural surplus materials. Rice and banana straw can be used to cultivate *Pleurotus ostreatus* and *P. sajor-caju* (Bonatti *et al.* 2004). In addition, many studies reported the use of agricultural waste for the cultivation of *P. eryngii*, also known as the King oyster mushroom. Sawdust, rice straw, rice bran, barley straw, wheat straw, and many other agricultural waste products have been used for the cultivation of *P. eryngii* (Kirbag & Akyüz 2008; Barshteyn & Krupodorova 2016; Jeznabadi *et al.* 2017). The use of agricultural waste to cultivate mushrooms has become a trend.

P. eryngii has excellent flavor, nutritional value, and health benefits (Jeznabadi *et al.* 2016). *P. eryngii* exerts antioxidative (He *et al.* 2016), anti-inflammatory (Lin *et al.* 2014; Chien *et al.* 2016), anti-aging (Zhang *et al.* 2021), hypoglycemic (Li *et al.* 2014), and immunopotentiation (Mariga *et al.* 2014) effects. *P. eryngii*, commonly grown in Taiwan, is a crucial economic crop. Although related studies have used different agricultural waste products to cultivate *P. eryngii*, no systematic study has examined the nutritional and functional benefits of using agricultural waste to cultivate *P. eryngii*. If scientific evidence proves that the quality of mushroom cultivated with agricultural wastes does not differ from that with sawdust, then the use of agrlicultual waste can be easily promoted.

Paddy straw and sugarcane bagasse are already used to grow King Oyster Musuroom years ago (Zhou *et al.* 2023). Accoring to the agricultural statistics of Ministry of Agriculture (https://agrstat.moa.gov.tw/sdweb/public/common/Download.aspx), the average surplus of paddy straw form 2014–2023 are 1,674,452 tons, and the average surplus of spent mushroom substrates from 2014–2023 are 167,771 tons. Paddy straw is the largest amount agricultural waste of agiculture. Spent mushroom substrates are also a big amount agricultural waste. *Phyllostachys makinoi* is the major bamboo species in Taiwan. There are approximately 1,245 million culms of bamboo in Taiwan, and 80% of them are *Phy. makinoi*. The utilization of bamboo forest are less than 1% recently, bamboo needs explore a new way to be used (Chen *et al.* 2024).

This study examined differences between *P. eryngii* cultivated using general sawdust and those cultivated using different agricultural materials such as paddy straw, sugarcane bagasse, bamboo crumb, spent *P. eryngii*, or *Flammulina velutipes* mushroom substrates, and other agricultural-derived products. In addition, this study analyzed the nutrients, extract content, total triterpenoids, total polysaccharides, ergosterol, and ergothioneine of *P. eryngii* cultivated in different formulas. The results of this study can be used as reference and evidence for promoting agricultural wastes to replace sawdust in the cultivation of *P. eryngii*.

MATERIALS AND METHODS

Chemical materials

Oleanolic acid, ergosterol and ergothioneine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile were purchased from Honeywell Burdick & Jackson

(Muskegon, MI, USA). All of the other chemicals used in this study were of analytical grades and were obtained commercially.

Spawn preparation

The strain of *P. eryngii*, provided by the Mushroom Laboratory of Taiwan Agricultural Research Institute, was cultured on potato dextrose agar (PDA; Difco, Sparks, MD, USA) medium at 24℃ without light, and was subcultured every 2 wk. The spawn was prepared with 85% sawdust, 14% rice bran, and 1% calcium carbonate (w/w, dry weight) followed by adjusting the water content of the mixture to about 65%. The mixture was filled to polypropylene plastic bottles (about 500 grams per bottle) before the bottles being autoclaved (121℃, 1.2 kg cm^{-2}) for 1 h. The bottles were then cooled at 20 ℃ till the center temperature of mixture in the bottle was below 30℃ before each bottle was inoculated with 1 dish of *P. erymgii* mycelia agar. The spawns were incubated at 24℃ till the mixture substrate was colonized fully by mycelium.

Sawdust and spent agricultural wastes collection

Six materials were collected. Sawdust was obtained from You-Cheng Lai (Guoxing, Nantou, Taiwan). Bamboo (*Phy. makinoi*) crumb was obtained from Chingsui Bamboo and Wood Shop (Zhushan, Nantou, Taiwan). Paddy straw and sugarcane crumb were obtained from Chin Yi Yang Development CO., Ltd. (Huwei, Yunlin, Taiwan). Spent *F. velutipes* substrate was obtained from Dewang Enoki Mushroom Farm (Wufeng, Taichung, Taiwan). Spent *P. eryngii* substrate was obtained from Huang's Farm (Wufeng, Taichung, Taiwan). Before substrate preparation, all sawdust and spent agricultural wastes were stacked and rinsed to adjust the water content of them to about 50%.

Substrate preparation, inoculation, incubation and harvest

The substrates were prepared as shown in Table 1 before the water content of the mixture

Table 1. The material contents of each formula used in culturing *Pleurotus eryngii*.

Formulas[z]	Material contents[y] (%)					
	Sawdust	Bamboo crumb	Paddy straw crumb	Sugarcane crumb	Spent *P. eryngii* substrate	Spent *Flammulina velutipes* substrate
P_{SA} (Sawdust)	59.0	0	0	0	0	0
P_{SA+BC} (Sawdust + bamboo crumb)	29.5	29.5	0	0	0	0
P_{SA+PSC} (Sawdust + paddy straw crumb)	29.5	0	29.5	0	0	0
P_{SA+SC} (Sawdust + sugarcane crumb)	29.5	0	0	29.5	0	0
P_{SA+PE} (Sawdust + spent *P. eryngii* crumb)	29.5	0	0	0	29.5	0
P_{SA+FV} (Sawdust + spent *F. veltipes* substrate)	29.5	0	0	0	0	29.5
P_{BC} (Bamboo crumb)	0	59.0	0	0	0	0
P_{PSC} (Paddy straw crumb)	0	0	59.0	0	0	0
P_{SC} (Sugarcane crumb)	0	0	0	59.0	0	0
P_{PE} (Spent *P. eryngii* substrate)	0	0	0	0	59.0	0
P_{FV} (Spent *F. velutipes* substrate)	0	0	0	0	0	59.0

[z] All formulas were amended using 15% rice bran, 25% wheat bran, and 1% $CaCo_3$ based on dry weight.
[y] The contents were based on dry weight.

was adjusted to about 65%. The mixture was filled to polypropylene plastic bags (about 1,000 grams per bag) which were then autoclaved (121℃, 1.2 kg cm^{-2}) for 1 h. The autoclaved bags were cooled at 20℃ till the center temperature of mixture in the bag below 30℃ before two spoonful of *P. erymgii* mycelia sawdust spawn were inoculated into each bag. The inoculated bags were incubated at 22℃ under dark till the mixture substrate was colonized fully by the mycelium.

Once the substrates were totally covered with the mycelium, bags were moved to the growth room followed by opening the top of each bag for exposing the surface of top-sided substrates. The bags were maintained at 13℃ under a relative humidity of 90–95% for 2 d (with light 8 h d^{-1}). Two days later, the room temperature was adjusted to 18℃ with the relative humidity adjusted to 88–90% till the fruiting bodies of *P. eryngii* formed. Once the fruiting bodies started to form, the room temperature were adjusted to 16℃ and the relative humidity adjusted to 85–88% throughout the harvest of the fruiting bodies.

The fruiting bodies were ready for harvest when the gills of fruiting body were formed and the diameter of caps were close to that of stipes. After harvest the fruiting bodies were immediately frozen and prepared for freeze-drying.

The mushrooms generated according to the above-mentioned eleven substrate cultivations were abbreviated as follows: (1) *P. eryngii* cultivated with only sawdust (P_{SA}), (2) *P. eryngii* cultivated with sawdust + bamboo crumb (P_{SA+BC}), (3) *P. eryngii* cultivated with sawdust + paddy straw crumb (P_{SA+PSC}), (4) *P. eryngii* cultivated with sawdust + sugarcane crumb (P_{SA+SC}), (5) *P. eryngii* cultivated with sawdust + spent *P. eryngii* substrate (P_{SA+PE}), (6) *P. eryngii* cultivated with sawdust + spent *F. velutipes* substrate (P_{SA+FV}), (7) *P. eryngii* cultivated with only bamboo crumb (P_{BC}), (8) *P. eryngii* cultivated with only paddy straw crumb (P_{PSC}), (9) *P. eryngii* cultivated with only sugarcane crumb (P_{SC}), (10) *P. eryngii* cultivated with only spent *P. eryngii* substrate (P_{PE}), and (11) *P. eryngii* cultivated with only spent *F. velutipes* substrate (P_{FV}).

Nutritional content analysis

The moisture was determined according to National Standards of the Republic of China (CNS) 5033 (Methods of Test for Moisture in Food). The ash content was determined according to CNS 5034 (Method of Test for Ash in Food). The crude protein content was determined according to CNS 5035 (Methods of Test for Crude Protein in Food). The crude fat content was determined according to CNS 5036 (Methods of Test for Crude Fat in Food). The carbohydrate content was calculated using the following formula: Carbohydrates (%) = 100 – (moisture + ash + crude lipid + crude protein).

Water-soluble extract and dilute ethanol-soluble extract contents

The water extract content was determined according to Taiwan Herbal Pharmacopeia 3rd Edition. Two grams of the dried product of a *P. eryngii* fruiting body was weighed and placed in an Erlenmeyer flask and 70 mL of water was added in it. The solution was shaken and soaked over 5 h (alternating shake and stand 30 min each for 5 h continuously); it was then allowed to stand for 16 h. The solution was filtered and the filtrate was diluted with water to 100 mL. Fifty milliliters of the filtrate was accurately measured and poured into an evaporating dish to evaporate and dry in a water bath. Then the evaporating dish was placed in an oven at 105℃ for 4 h before being cooled in a desiccator. The water extract content (%) was calculated as follows: Water extract (%) = (weight of the evaporating dish after drying − weight of the empty evaporating pan)/weight of the examined sample × [1 − weight loss value of dry sample (%)] × 2 × 100%.

Total polysaccharide detection

The determination of water-soluble crude polysaccharide content was performed using the phenol-sulfuric acid method, which was modified according to the method of Dubois *et al.*

(1956). A total of 0.5 g of *P. eryngii* sample was added 3 times its volume of 80% ethanol and then the solution was heated at 75℃ for 6 h to remove the fat. The residue was added with water to obtain a solid-liquid ratio of 1 : 20. The mixture was heated at 95℃ and extracted for 150 min. Then, 80% ethanol was added, and the solution was filtered after precipitating at 4℃ for 24 h. The precipitate was dried and weighed to obtain the total water-soluble crude polysaccharide. Polysaccharide sample solution was prepared at a concentration of 50 ppm. Two milliliters of the sample solution was mixed evenly in 1 mL of 5% phenol solution before 5 mL of concentrated sulfuric acid was quickly added. The mixed solution was shaken for 30 s, stood at room temperature for 10 min to allow the reaction to proceed fully and then placed in a water bath for 20 min. The absorbance of 1 mL of the reaction solution was measured at 490 nm by using a spectrophotometer. Glucose was used for the construction of the standard curve.

Total triterpenoid content detection

A total of 2 g of *P. eryngii* sample powder was accurately weighed. Then, 100 mL of ethyl acetate was added and the mixture was ultrasonically shaken for 30 min. The filtrate was filtered and quantified with ethyl acetate to 100 mL as the test liquid. A total of 0.3 mL of the test solution was placed in test tubes and evaporated to dry in a 70℃ water bath (only the solute was retained). After the test tubes were removed and cooled, 0.3 mL of 5% vanillin-glacial acetic acid (w/v) and 1.0 mL of perchloric acid were added to each test tube in sequence. After the test tubes were sealed, they were placed in a 70℃ water bath and heated for 25 min. After the reaction completed, the mixture was immediately cooled with ice water for 3 min. Finally, 10.0 mL of glacial acetic acid was added; the test tubes were placed on a shaker at room temperature, and their absorbance was measured at 550 nm by using a spectrophotometer. Oleanolic acid was used for the construction of the standard curve (Cai *et al*. 2019).

High-performance liquid chromatography (HPLC) analysis of *P. eryngii* samples

The ergosterol standard solution was prepared by dissolving 0.5 mg of ergosterol standard in 1 mL of methanol. The ergothioneine standard solution was prepared by dissolving 1 mg of ergothioneine standard in 1 mL of 50% methanol. To prepare the betulinic acid standard solution, 0.5 mg betulinic acid standard was dissolved in 1 mL of methanol. The *P. eryngii* test product solution was prepared as follows: 2 g of *P. eryngii* test product powder was accurately weighed, and 30 mL of 50% methanol was added. The mixture was ultrasonically shaken for 30 min and filtered. The remaining residue was extracted again by repeating the previous steps. The filtrates were combined, concentrated, redissolved to 10 mL with 50% methanol, and filtered using a 0.45-μm microporous membrane. The sample was analyzed using HPLC.

The chromatography fingerprint was analyzed using HPLC equipment (10AVP HPLC System, Shimadzu, Kyoto, Japan), and its chromatography column was a COSMOSIL 5C18-AR-II column (5 μm, 4.6 mm inside diameter (ID) × 250 mm). The mobile phase consisted of methanol (A) and 0.1% aqueous formic acid (B) using a gradient elution of 1% A at 0–10 min, 1–30% A at 10–20 min, 30–40% A at 20–30 min, and 40–95% A at 30–60 min. The flow rate was 1.0 mL per minute, and the detection wavelength was 280 nm for ergosterol, 254 nm for ergothioneine, and 210 nm for betulinic acid to observe the fingerprint spectrum and peaks of specific components. The analysis was performed at room temperature. The injection volume was 20 μL and analysis time was 60 min.

Ergosterol was analyzed using a COSMOSIL 5C18-AR-II column (5 μm, 4.6 mm ID × 250 mm) as the chromatography column and methanol as the mobile phase at a flow rate 1.0 mL per minute. The detection wavelength was 280 nm and the temperature was room temperature. The test sample injection volume

was 20 μL and the analysis time was 20 min. Ergothioneine was analyzed by a COSMOSIL HILIC column (5 μm, 4.6 mm ID × 250 mm). The mobile phase was acetonitrile and water (90 : 10). The flow rate was 1.0 mL per minute, and the detection wavelength was 254 nm. The temperature was at room temperature, the test product injection volume was 10 μL, and the analysis time was 30 min.

Statistical analysis

All the assays were carried out in triplicate and all experimental data are presented as mean ± standard deviation (*SD*). Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test (IBM SPSS Statistics 25 software). The criterion for statistical significance in this study was $P < 0.05$. Histograms were drawn with Sigmaplot 14 software.

Table 2. Comparisons of *Pleurotus eryngii* growth based on yield and biological efficiency.

	P. eryngii	
Formulas[z]	Yield (g bag^{-1})	Biological efficiency (%)
P_{SA}	275.94 ± 21.48 b[y]	69.89 ± 5.22 d
P_{SA+BC}	252.69 ± 21.79 d	62.53 ± 5.46 e
P_{SA+PSC}	262.17 ± 18.65 c	100.60 ± 7.61 a
P_{SA+SC}	282.85 ± 17.49 b	74.83 ± 4.95 c
P_{SA+PE}	210.98 ± 13.89 e	55.24 ± 3.58 f
P_{SA+FV}	191.02 ± 20.08 f	51.16 ± 5.30 g
P_{BC}	219.10 ± 19.28 e	56.18 ± 4.89 f
P_{PSC}	211.13 ± 24.82 e	67.81 ± 8.06 d
P_{SC}	323.79 ± 27.64 a	90.91 ± 7.84 b
P_{PE}	120.75 ± 22.22 g	30.57 ± 5.66 i
P_{FV}	146.02 ± 11.21 h	41.08 ± 3.44 h

[z] All formulas were amended using 15% rice bran, 25% wheat bran, and 1% $CaCO_3$ based on dry weight. Biological efficiency (%) = the weight of fresh mushroom harvested/dry matter content of substrate before inoculation × 100%.

[y] Data were presented as the mean ± *SD* ($n = 48$, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

RESULTS

Yields and biological efficiency

Table 2 showed the yields and biological efficiency of *P. eryngii* cultured with different recycled agricultural wastes. The results indicated that the yield of P_{SC} was the highest (323.79 ± 27.64 g bag^{-1}), fallowed by P_{SA+SC}, P_{SA}, P_{SA+PSC}, P_{SA+BC}, P_{BC}, P_{SA+PE}, P_{PSC}, P_{SA+FV}, P_{FV}, and P_{PE}. The biological efficiency was defined as the numbers of substrates that could efficiently be transformed to produce fruiting bodies of *P. eryngii*. The biological efficiency of P_{SA+PSC} was the highest (100.60 ± 7.61%), fallowed by P_{SC}, P_{SA+SC}, P_{SA}, P_{PSC}, P_{SA+BC}, P_{BC}, P_{SA+PE}, P_{SA+FV}, P_{FV}, and P_{PE}. The biological efficiency of formula P_{SA+BC}, P_{SA+PSC}, P_{SA+PSC}, P_{PSC}, and P_{SC} were all higher than 60%. The major substrates in these formulas were potential to be used well by *P. eryngii*.

Nutritional analysis

As shown in Table 3, in each group of *P. eryngii*, the moisture content was between 87.1% and 90.4%, the crude ash content was between 0.65% and 0.91%, the crude protein content was between 1.93% and 4.00%, the crude fat content was not detected, and the carbohydrate content was between 6.94% and 8.21%. Whether bamboo crumb, paddy straw crumb, sugarcane crumb, spent *P. eryngii* substrate, or spent *F. velutipes* substrate was used to completely or partially replace sawdust, no significant difference was observed in terms of moisture, crude fat, or carbohydrate content of the cultivated *P. eryngii*. The crude ash contents of P_{PE} or P_{FV} were significantly higher than that of P_{SC}, but there was no difference compared with that of P_{SA}.

Contents of the diluted ethanol-soluble extract and the water-soluble extract

The contents of the diluted ethanol-soluble and water-soluble extracts were determined to examine the content of components that can be dissolved in diluted ethanol (50% ethanol) or water. This method is a standard for quality control in traditional Chinese medicine and is used to compare samples cultivated in differ-

Table 3. Nutrient content of *Pleurotus eryngii* cultivated in sawdust or different agricultural surplus materials.

	Nutrient contents^z				
Formulas	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
P_{SA}	89.5 ± 1.61 a^y	0.84 ± 0.03 ab	2.41 ± 0.18 bc	N.D.	7.33 ± 0.55 a
P_{SA+BC}	88.6 ± 2.83 a	0.74 ± 0.12 ab	2.52 ± 0.13 b	N.D.	8.21 ± 0.49 a
P_{SA+PSC}	90.4 ± 1.97 a	0.72 ± 0.04 b	1.93 ± 0.11 c	N.D.	7.02 ± 0.71 a
P_{SA+SC}	89.7 ± 1.84 a	0.74 ± 0.13 ab	2.22 ± 0.24 bc	N.D.	7.44 ± 0.73 a
P_{SA+PE}	88.6 ± 1.46 a	0.72 ± 0.07 ab	2.69 ± 0.13 b	N.D.	8.03 ± 0.19 a
P_{SA+FV}	89.1 ± 2.97 a	0.82 ± 0.07 ab	2.91 ± 0.31 b	N.D.	7.17 ± 0.22 a
P_{BC}	89.9 ± 1.41 a	0.72 ± 0.01 b	2.54 ± 0.12 b	N.D.	6.94 ± 0.52 a
P_{PSC}	89.4 ± 1.80 a	0.71 ± 0.03 b	2.61 ± 0.07 b	N.D.	7.28 ± 0.63 a
P_{SC}	90.0 ± 2.21 a	0.65 ± 0.04 b	2.29 ± 0.13 bc	N.D.	7.10 ± 0.45 a
P_{PE}	87.1 ± 2.09 a	0.91 ± 0.08 a	3.92 ± 0.09 a	N.D.	8.08 ± 0.33 a
P_{FV}	87.6 ± 2.93 a	0.81 ± 0.09 ab	4.00 ± 0.15 a	N.D.	7.62 ± 0.37 a

^{z} All examined products were in fresh form.

^{y} Data were presented as the mean ± *SD* (*n* = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). N.D. means not detected. Different letters represented significant differences at $P < 0.05$.

ent media and examine differences between products cultivated using different substrates. As shown in Table 4, the contents of diluted ethanol-soluble and water-soluble extracts in *P. eryngii* samples were 38.2–42.8% and 43.6–57.5%, respectively. From the results, it could be seen that no significant differences were observed in the contents of diluted ethanol-soluble extracts between P_{SA} and the other products. However, the water-soluble extracts of P_{PE} were higher than those of P_{SA}. Many factors such as temperature, humidity, and climate as well as the cultivation method used can cause changes in the content of these extracts.

Table 4. Contents of diluted ethanol-soluble and water-soluble extracts of mushrooms cultivated using sawdust or different agricultural surplus materials

	Contents^z	
Formulas	Diluted ethanol-soluble extract (%)	Water-soluble extract (%)
P_{SA}	39.8 ± 0.2 a^y	45.3 ± 0.9 bc
P_{SA+BC}	42.2 ± 0.2 a	47.8 ± 0.5 bc
P_{SA+PSC}	42.2 ± 2.1 a	46.2 ± 1.6 bc
P_{SA+SC}	39.8 ± 0.5 a	46.5 ± 2.2 bc
P_{SA+PE}	39.5 ± 1.3 a	43.6 ± 2.1 c
P_{SA+FV}	40.8 ± 0.9 a	50.9 ± 2.1 b
P_{BC}	40.9 ± 1.4 a	43.7 ± 1.7 c
P_{PSC}	40.2 ± 1.1 a	47.1 ± 2.5 bc
P_{SC}	38.2 ± 0.9 a	46.3 ± 0.8 b
P_{PE}	42.8 ± 0.8 a	57.5 ± 2.3 a
P_{FV}	41.6 ± 0.8 a	50.6 ± 2.2 b

^{z} Results presented as grams of liquid extract per 100 g. All examined products were in powder form.

^{y} Data were presented as the mean ± *SD* (*n* = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

Determination of total triterpenoids and total polysaccharides

The total triterpenoid content was based on the content of oleanolic acid per gram (mg oleanolic acid g^{-1}), and the content of total polysaccharides was mostly based on the content of glucose per gram (mg glucose g^{-1}). As shown in Table 5, the content of total triterpenoids in *P. eryngii* samples was between 1.85 and 2.30 mg oleanolic acid g^{-1}, and that of total polysaccharides was between 1.07 and 4.30 mg glucose g^{-1}. No significant difference in the total triterpenoid content was observed between P_{SA} and the other products. Whereas, the total polysaccharide content was higher in P_{SC} and lower in P_{SA+BC}, P_{SA+FV}, P_{BC}, and P_{PE} than P_{SA}.

Table 5. Total triterpenoid and total polysaccharide contents in mushrooms cultivated using sawdust or different agricultural surplus materials.

	Contents[z]	
Formulas	Total triterpenoids ($mg\ g^{-1}$)	Total polysaccharides ($mg\ g^{-1}$)
P_{SA}	1.94 ± 0.08 a[y]	2.70 ± 0.08 b
P_{SA+BC}	1.87 ± 0.13 a	1.07 ± 0.08 d
P_{SA+PSC}	1.85 ± 0.12 a	2.19 ± 0.18 bc
P_{SA+SC}	2.30 ± 0.33 a	2.04 ± 0.25 bc
P_{SA+PE}	2.16 ± 0.14 a	2.28 ± 0.05 bc
P_{SA+FV}	1.96 ± 0.12 a	1.28 ± 0.28 d
P_{BC}	2.19 ± 0.64 a	1.38 ± 0.07 d
P_{PSC}	2.14 ± 0.19 a	2.30 ± 0.34 bc
P_{SC}	1.93 ± 0.06 a	4.30 ± 0.32 a
P_{PE}	2.03 ± 0.18 a	1.95 ± 0.11 c
P_{FV}	2.11 ± 0.24 a	2.41 ± 0.03 b

[z] Total triterpenoids in milligrams of oleanolic acid per gram. Total polysaccharides in milligrams of glucose per gram. All examined products were in powder form.

[y] Data were presented as the mean ± *SD* ($n = 8$, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

Analysis and comparison of the HPLC fingerprint of *P. eryngii*

As shown in Fig. 1A, the retention times of the three index components of ergothioneine, betulinic acid, and ergosterol were 3.15, 16.16, and 42.26 min, respectively. The presence of ergothioneine, ergosterol, and betulinic acid was observed in fingerprint chromographs by comparing the results of mushrooms cultivated in sawdust and in other agricultural surplus materials. In addition, the peak patterns of the fingerprints were found to be consistent (Figs. 1B, 1C, and 1D). The composition of *P. eryngii* grown using bamboo crumb, paddy straw crumb, sugarcane crumb, spent *P. eryngii* substrate, and spent *F. velutipes* substrate formulations was the same as that of *P. eryngii* grown using sawdust.

Analysis of ergosterol and ergothioneine

HPLC was used to analyze the content of ergosterol and ergothioneine in *P. eryngii* samples cultivated using sawdust or different agricultural surplus materials in this study. At a wavelength of 280 nm, the retention time of ergosterol was approximately 13.63 min. The retention time of ergothioneine at a wavelength of 254 nm was approximately 24.25 min (Fig. 2). The regression curve equation of the calibration curves revealed a strong linear relationship at a concentration of 3.1–200 $\mu g\ mL^{-1}$ for ergosterol and 5.0–50 $\mu g\ mL^{-1}$ for ergothioneine (data not shown). As shown in Table 6 and Fig. 3, the ergosterol content was between 0.015 and 0.067 $mg\ g^{-1}$ and the ergothioneine content was between 0.890 and 2.542 $mg\ g^{-1}$. The contents of ergosterol were higher in P_{SA+PSC} and P_{PE} than in P_{SA}, but that was lower in P_{SA+PE} than in P_{SA} (Table 6 and Fig. 3). On the other hand, the content of ergothioneine in P_{SA+BC}, P_{SA+FV} and P_{FV} were higher than in P_{SA}, but that in P_{SA+PSC}, P_{SA+SC}, P_{SA+PE}, P_{PSC}, P_{SC} and P_{PE} was lower than that in P_{SA} (Table 6 and Fig. 3).

DISCUSSIONS

Generally, sawdust from freshly cut trees is used as the medium to cultivate commercially available *P. eryngii* (Ohga 2000). The cultivation of mushrooms with mediums other than sawdust such as recycled agricultural wastes is an environmentally friendly approach; however, scientific evidence must be obtained to prove that their production capacity and quality. In this study, we compared the differences between *P. eryngii* cultivated using general sawdust and that cultivated using different agricultural materials.

As showed in Table 2, the yield and biological efficiency of formula P_{SC} and P_{SA+SC} were higher than those of P_{SA}. Even if the sawdust contents of formula was replaced a half percent by sugarcane crumb, the yield and biological efficiency of P_{SA+SC} were also higher than that of P_{SA}. The data showed that sugarcane crumb was better than sawdust for *P. eryngii* cultivation. The yields of formula P_{SA+BC} and P_{SA+PSC} were a little less than that of P_{SA}. The yields of formula P_{BC} and P_{PSC} were much less than that of P_{SA}. It

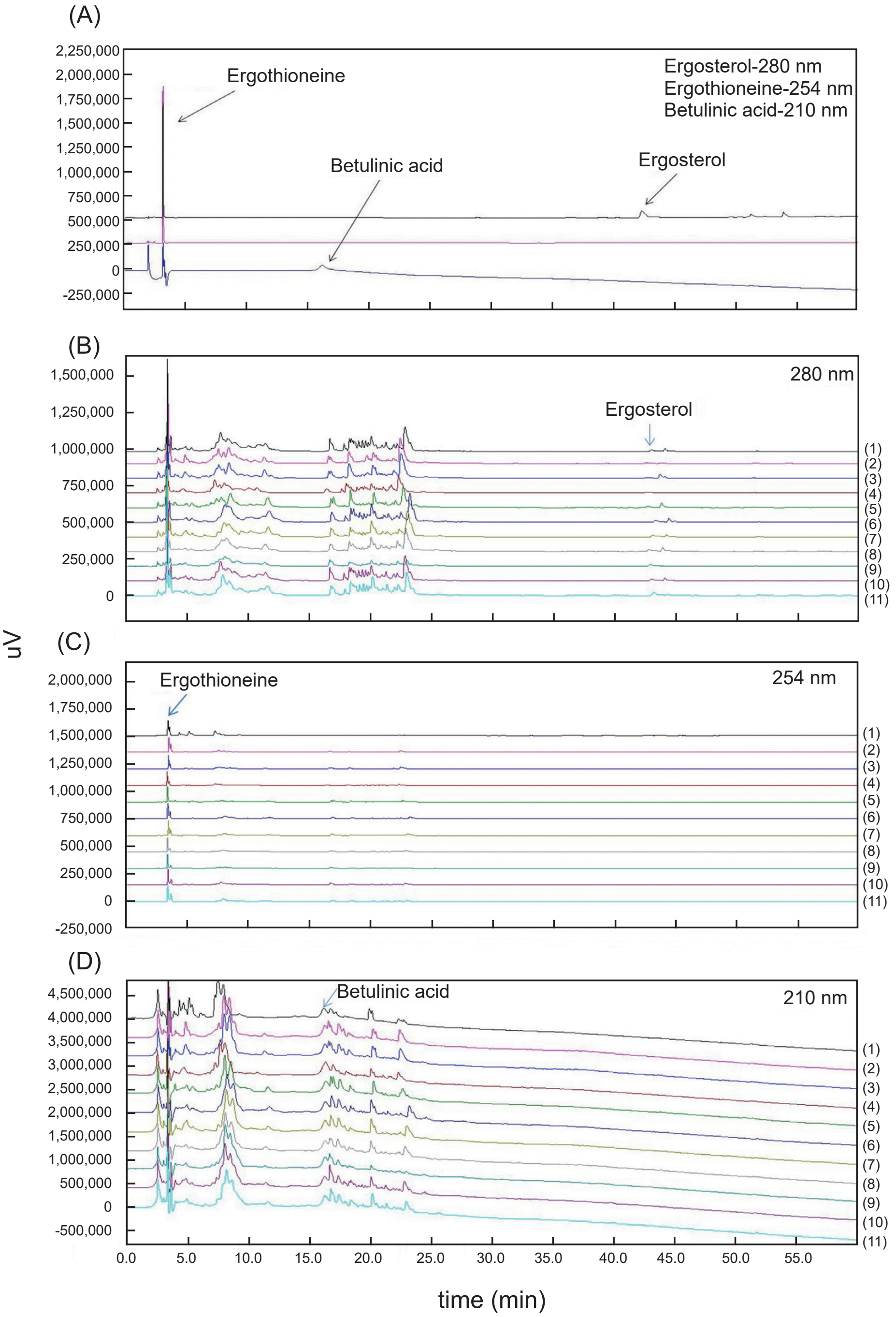

Fig. 1. Comparison of HPLC fingerprints of king oyster mushrooms cultivated using sawdust or agricultural surplus materials at different wavelengths. (A) Standards of ergosterol, ergothioneine, and betulinic acid at (B) 280 nm, (C) 254 nm, and (D) 210 nm. (1) P_{SA}: *Pleurotus eryngii* cultivated with sawdust; (2) P_{SA+BC}: *P. eryngii* cultivated with sawdust + bamboo crumb; (3) P_{SA+PSC}: *P. eryngii* cultivated with sawdust + paddy straw crumb; (4) P_{SA+SC}: *P. eryngii* cultivated with sawdust + sugarcane crumb; (5) P_{SA+PE}: *P. eryngii* cultivated with sawdust + spent *P. eryngii* substrate; (6) P_{SA+FV}: *P. eryngii* cultivated with sawdust + spent *F. velutipes* substrate; (7) P_{BC}: *P. eryngii* cultivated with bamboo crumb; (8) P_{PSC}: *P. eryngii* cultivated with paddy straw crumb; (9) P_{SC}: *P. eryngii* cultivated with sugarcane crumb; (10) P_{PE}: *P. eryngii* cultivated with spent *P. eryngii* substrate; and (11) P_{FV}: *P. eryngii* cultivated with spent *F. velutipes* substrate.

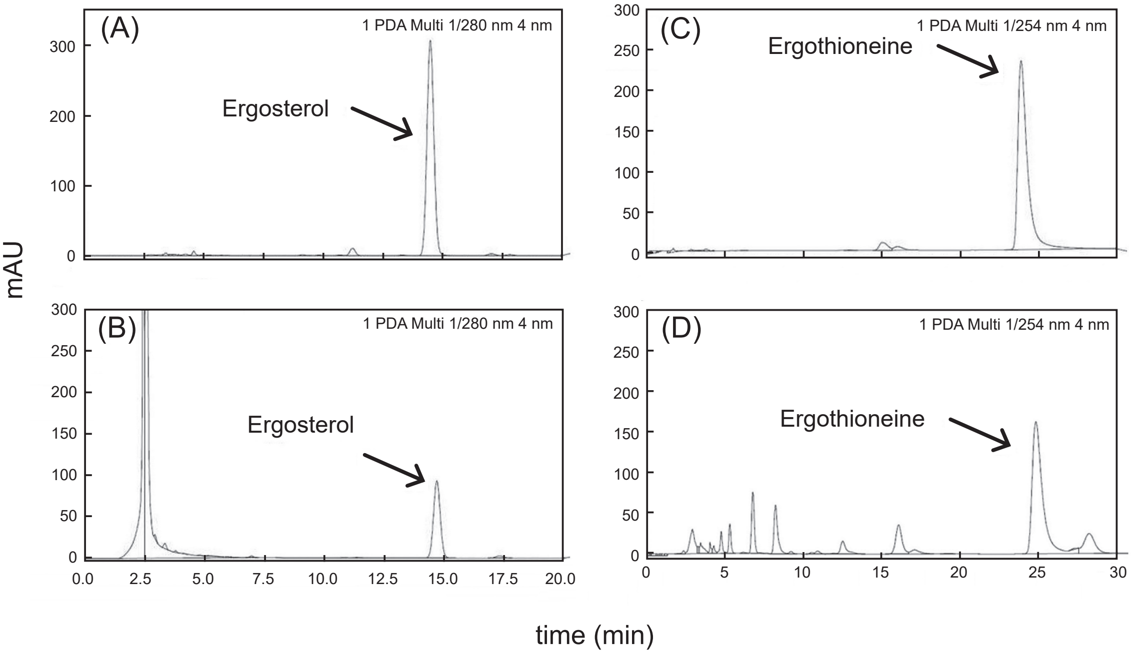

Fig. 2. HPLC chromatograms of ergosterol, ergothioneine and *Pleurotus eryngii*. (A) standard (ergosterol) and (B) *P. eryngii* at a wavelength of 280 nm; (C) standard (ergothioneine) and (D) *P. eryngii* at a wavelength of 254 nm.

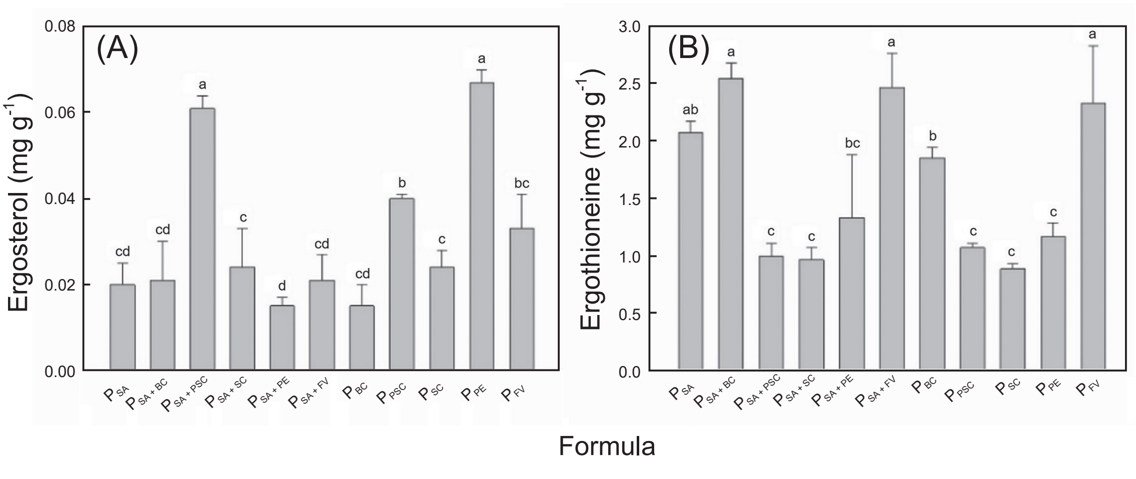

Fig. 3. Comparisons of ergosterol (A) and ergothioneine (B) contents of *Pleurotus eryngii* cultivated using sawdust or agricultural surplus materials. All values were expressed as the mean ± *SD* (n = 8, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$. P_{SA}: *P. eryngii* cultivated with sawdust; P_{SA+BC}: *P. eryngii* cultivated with sawdust + bamboo crumb; P_{SA+PSC}: *P. eryngii* cultivated with sawdust + paddy straw crumb; P_{SA+SC}: *P. eryngii* cultivated with sawdust + sugarcane crumb; P_{SA+PE}: *P. eryngii* cultivated with sawdust + spent *P. eryngii* substrate; P_{SA+FV}: *P. eryngii* cultivated with sawdust + spent *Flammulina velutipes* substrate; P_{BC}: *P. eryngii* cultivated with bamboo crumb; P_{PSC}: *P. eryngii* cultivated with paddy straw crumb; P_{SC}: *P. eryngii* cultivated with sugarcane crumb; P_{PE}: *P. eryngii* cultivated with spent *P. eryngii* substrate; and P_{FV}: *P. eryngii* cultivated with spent *F. velutipes* substrate.

Table 6. Ergosterol and ergothioneine contents of mushrooms cultivated using sawdust or agricultural surplus materials.

	Contents[z]	
Formulas	Ergosterol (mg g^{-1})	Ergothioneine (mg g^{-1})
P_{SA}	0.020 ± 0.005 cd[y]	2.074 ± 0.099 b
P_{SA+BC}	0.021 ± 0.009 cd	2.542 ± 0.131 a
P_{SA+PSC}	0.061 ± 0.003 a	0.998 ± 0.116 d
P_{SA+SC}	0.024 ± 0.009 c	0.968 ± 0.108 d
P_{SA+PE}	0.015 ± 0.002 d	1.333 ± 0.545 c
P_{SA+FV}	0.021 ± 0.006 cd	2.462 ± 0.297 a
P_{BC}	0.015 ± 0.005 cd	1.854 ± 0.088 b
P_{PSC}	0.040 ± 0.001 b	1.074 ± 0.041 cd
P_{SC}	0.024 ± 0.004 c	0.890 ± 0.047 d
P_{PE}	0.067 ± 0.003 a	1.166 ± 0.123 cd
P_{FV}	0.033 ± 0.008 bc	2.327 ± 0.497 a

[z] All examined products were in powder form.

[y] Data were presented as the mean ± *SD* ($n = 8$, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test). Different letters represented significant differences at $P < 0.05$.

showed that bamboo crumbs and paddy straw crumbs were not as good as sawdust for *P. eryngii* cultivation. Although Sardar *et al.* (2017) suggested that cellulose improve yield of *P. eryngii*, cellulose in sawdust was not as rich as that in bamboo and paddy straw, the yield of formula P_{SA} in this study was much higher than that of formula P_{BC} and P_{PSC}. Table 2 showed that adding sawdust to the formulas could improve the yields when the bamboo crumbs or paddy straw crumbs were used for cultivating of *P. eryngii*. On the contrary, a result of Hassan *et al.* (2010) showed that the yield from sawdust formula was much higher than that from sugarcane or paddy straw formula. Previous studies suggested that cellulose and lignin contents are not related to the yield of fruiting bodies of *P. eryngii* (Philippoussis *et al.* 2001; Atila 2017). The results in this research coincided with reports by Philippoussis *et al.* (2001) and Atila (2017) that the yield of *P. eryngii* was not correlated with the content of lignin, cellulose, or hemicellulose in the cultivation formula. Maybe different strains of *P. eryngii* prefer different contents of lignin or cellulose constructs or some special compounds in different formula. And *P. eryngii* strain used in this study prefer sawdust to bamboo and paddy straw. So the biological efficiency of formula P_{BC} and P_{PSC} were a little lower than that of P_{SA}. But adding sawdust to the formulas could improve the biological efficiency when the bamboo crumbs or paddy straw crumbs were used for cultivating of *P. eryngii*.

The yields of formula P_{PE} and P_{FV} were much lower than that of P_{SA}. It showed that spent *P. eryngii* substrate and spent *F. velutipes* substrate were not as good as fresh sawdust for *P. eryngii* cultivation. However adding fresh sawdust to the formulas could improve the yields when the spent *P. eryngii* substrate and spent *F. velutipes* substrate were used for cultivating of *P. eryngii*. Spent *P. eryngii* substrate and spent *F. velutipes* substrate in this test were all composted over 2 mo. In this period, perhaps the nutrient suitable for *P. eryngii* could have been broked down that led to perhaps produced some bad secondary metabolites unsuitable for *P. eryngii*. Philippoussis *et al.* (2001) showed that high yield of *P. eryngii* was the results of high carbon/nitrogen ratio formula (Philippoussis *et al.* 2001) and that could explain why the yield of the formula P_{SA+PE} was much higher than that of the formula P_{PE}, and the yield of the formula P_{SA+FV} was much higher than that of the formula P_{FV}.

The crude protein contents of P_{PE} and P_{FV} were higher than that of P_{SA}. The precise study described that *P. eryngii* and *F. velutipes* are rich in protein (Reis *et al.* 2012). There were also literatures pointing out that the nitrogen content of spent *P. eryngii* substrate was higher than that of fresh sawdust (Chen *et al.* 2013). The high nitrogen content of spent *P. eryngii* substrate or spent *F. velutipes* substrate may cause the protein content of P_{PE} or P_{FV} to be higher than that of P_{SA}. However, the type of protein in *P. eryngii* cultivated in spent mushroom substrate and whether its nutritional value has any effect should be studied in the

future. Taken together, the nutrient contents of *P. eryngii* cultivated from other matrix was close to that of from fresh sawdust, therefore using agricultural waste to completely or partially replace fresh sawdust to cultivate *P. eryngii* is a feasible method for environmental protection and economical growth.

Triterpenoids and polysaccharides are the functional components of *P. eryngii*. They exhibit antioxidative activity (Xue *et al*. 2015) and prevent hyperlipidemia and metabolic syndrome associated with obesity (Zhao *et al*. 2020); thus, they can be used as the standard for the content of functional components. The results showed that the total polysaccharide content was higher in P_{SC} and lower in P_{SA+BC}, P_{SA+FV}, P_{BC}, and P_{PE} than P_{SA}. The high sugar content may be attributable to the high sugar content in the substrate that led to higher polysaccharides in P_{sc}. But, according to the report of Abd El-Zaher *et al*. (2022), polysaccharides contents produce form *P. eryngii* cultivated of wheat straw, rice straw and sugarcane bagasse are so close (0.6–0.7 mg mL^{-1}). This is an interesting issue could be further study later.

In this study, HPLC fingerprints were used to compare the differences in mushrooms cultivated in different materials. The results indicated that the peak patterns of the fingerprints were found to be consistent. There had no different components and the difference only observed in the content of the components. Therefore, the use of agricultural waste to cultivate *P. eryngii* should be considered as a safe and feasible method. On the other hasnd, ergosterol and ergothioneine are crucial active components of *P. eryngii* that exert antioxidative and anti-inflammatory effects (Liang *et al*. 2013; Kawai *et al*. 2014). Ergosterol in mushrooms was a new focus for its medical potential in recent years. Ergosterol, a plant sterol known to have hypolipidemic and antitumor functions (Yazawa *et al*. 2000; Takaku *et al*. 2001; Hu *et al*. 2006) is well-recognized in *Pleurotus* mushrooms. It is a precursor of vitamin D2 (provitamin) which is converted to vitamin D2 by ergosterol under UV irradiation (Kalač 2013). Ergothioneine was synthesized only from fungi and mycobacterium (Rodriguez Estrada *et al*. 2009). Liang *et al*. (2013) reported that *P. eryngii* were rich in ergothioneine. Ergothioneine is similar in many respects to glutathione (GSH). Many literatures have confirmed that it has antioxidant properties such as inhibiting lipid oxidation, scavenging free radicals, and peroxynitriting (Aruoma *et al*. 1999). Previous studies also indicated that ergothioneine can significantly increase superoxide dismutase (SOD) activity, glutathione/glutathione disulfide (GSH/GSSG) ratio, and reduce thiobarbituric acid reactive substances (TBARS) content in the brain that significantly reduces the damage of chemotherapy drug-induced brain tissue associted with learning and memory (Song *et al*. 2010). The ergosterol and ergothioneine could be measured in all tested products and could be used as indicators in *P. eryngii* quality control. In this study, the contents of ergosterol and ergothioneine in *P. eryngii* cultivated from different agricultural wastes were detected. As shown in Table 6, the contents of ergosterol extracted from *P. eryngii* cultivated with formula P_{SA+PSC} and P_{PE} were significant higher than those with others, but the data did not show any correlation to the formula. The contents of ergothioneine extracted from *P. eryngii* cultivated with formula P_{SA+BC}, P_{SA+FV} and P_{FV} were significant higher than those from others. The contents of ergothioneine extracted from *P. eryngii* cultivated with formula P_{SA} and P_{BC} were the second highest in concentration (Table 6). The data indicated the contents of ergothioneine extracted from *P. eryngii* were promoted with the additon of sawdust. The content of ergothioneine was correlated to the growth substrates of *P. eryngii*. Herein, the spent *F. velutipes* substrate exhibited itself as the best to promote ergothioneine in *P. eryngii* whereas the sawdust and bamboo crumb were the second best.

CONCLUSIONS

The results of this study indicated the sam-

ples grown using spent *P. eryngii* substrate or spent *F. velutipes* substrate have higher crude protein content that need further researches. The water-soluble extract contents of *P. eryngii* cultured with spent *P. eryngii* substrate was higher than that of *P. eryngii* cultured with sawdust, but there was no difference in the diluted ethanol extract contents. The total polysaccharides content of *P. eryngii* cultured with sugarcane substrate was significantly higher, while that cultured with bamboo crumb was lower, and there had no difference in the content of total triterpenoids. From the observable range of the fingerprint spectrum, it was seen that the composition types of *P. eryngii* cultured from any agricultural waste substrate were the same. The content of health ingredients ergosterol and ergothinione was different due to different cultivation materials. Overall, recycled materials such as bamboo crumb, paddy straw crumb, sugarcane crumb, spent *P. eryngii* substrate, and spent *F. velutipes* substrate can be used to completely or partially replace fresh sawdust to cultivate *P. eryngii* to effectively reduce agricultural waste. Especially sugarcane, paddy straw and bamboo crumb are potential substrates for King Oyster Mushroom cultivation.

FUNDING

This research was funded by the Taiwan Agricultural Research Institute, Ministry of Agriculture (grant number: 107AS-18.1.2-CI-C).

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不同農業廢棄物回收再利用培養杏鮑菇及其成分比較

李瑋崧 [1] 鄭閔謙 [2] 彭文煌 [3,*] 蔡仁傑 [4,*]

摘要

李瑋崧、鄭閔謙、彭文煌、蔡仁傑。2024。不同農業廢棄物回收再利用培養杏鮑菇及其成分比較。台灣農業研究 73(4):235–250。

本研究採用竹屑、稻草屑、甘蔗屑、杏鮑菇廢基質或金針菇廢基質全部或部分取代新鮮木屑來培養杏鮑菇。比較了營養成分與功能成分。比較指紋圖譜以及高效液相層析法 (high-performance liquid chromatography; HPLC) 分析的麥角固醇與麥角硫因的含量。結果表明，使用杏鮑菇廢基質或金針菇廢基質培養的樣本具有較高的粗蛋白含量。用杏鮑菇廢基質培養的杏鮑菇水溶性浸出物含量高於用新鮮木屑培養的杏鮑菇水溶性浸出物含量。甘蔗屑基質培養的杏鮑菇總多醣含量顯著高於新鮮木屑培養者，而竹屑培養的杏鮑菇總多醣含量較低。HPLC 指紋圖譜結果表明，從本試驗所用之任何農業廢棄物基質培養的杏鮑菇成分都是相同的。麥角固醇含量為 0.015–0.067 mg g^{-1}，麥角硫因含量為 0.890–2.542 mg g^{-1}。這些結果表明，使用回收農業廢棄資材代替木屑來種植杏鮑菇可能是一種安全且有價值的解決方案。

關鍵詞：杏鮑菇、農業廢棄物、營養、麥角固醇、麥角硫因。

投稿日期：2024 年 8 月 8 日；接受日期：2024 年 10 月 6 日。

[*] 通訊作者：jenchieh@mail.cmu.edu.tw; whpeng@mail.cmu.edu.tw
[1] 農業部農業試驗所植物病理組副研究員。臺灣 臺中市。
[2] 農業部畜產試驗所南區分所副研究員。臺灣 屏東縣。
[3] 中國醫藥大學中國藥學暨中藥資源學系教授。臺灣 臺中市。
[4] 中國醫藥大學藥學系副教授。臺灣 臺中市。

台灣農業研究 (J. Taiwan Agric. Res.) 73(4):251–258 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0003

研究報告

添加花粉對外米綴蛾 (*Corcyra cephalonica*) 產卵量及生活史的影響

吳嘉鴻[1] 賴成金[2] 洪心慈[1] 吳立心[3,*]

摘要

吳嘉鴻、賴成金、洪心慈、吳立心。2024。添加花粉對於外米綴蛾 (*Corcyra cephalonica*) 產卵量及生活史的影響。台灣農業研究 73(4):251–258。

生物防治是有害生物綜合管理中重要的方法，外米綴蛾 (*Corcyra cephalonica*) 為世界重要的倉儲害蟲，然而其新鮮卵粒卻能夠作為多種重要天敵寄生蜂最有效的代用寄主。本試驗以米糠、大豆粉及花粉為原料，依文獻與飼養經驗調配 4 種不同比例的配方，分別飼養外米綴蛾並測量 (1) 雌成蟲累積出現率；(2) 雌蟲百分比；(3) 雌蟲重；(4) 產卵量；(5) 百卵重；(6) 每毫升卵的生產成本，期能提升卵粒生產的質量。結果顯示以花粉 10% 配方飼養的外米綴蛾雌成蟲，不僅能縮短生活史且能提早羽化、其他生長參數亦優於其他處理，顯示花粉對外米綴蛾的大量飼養極具營養價值。然而比較每毫升卵粒的生產成本，添加花粉所需成本雖高於其他 3 配方，但每隻雌蛾成蟲的平均產卵量高達 157.19 ± 12 粒，與目前報導最佳的雌蛾產卵紀錄 166.63 粒接近。花粉富含多種維生素，確定為有效且經濟的外米綴蛾卵粒的添加物。未來將測試花粉有效的最低比例，以期應用於自動化生產，優化寄生蜂生物防治的完整產程。

關鍵詞：食物配方、花粉、外米綴蛾、產卵量、生活史評估。

前言

外米綴蛾 (*Corcyra cephalonica*) 是重要倉儲害蟲，但也被運用作為各種害蟲生物防治昆蟲天敵的重要代用寄主，例如其卵粒被運用為赤眼卵寄生蜂 (*Trichogramma* spp.) 的代用寄主，也是捕食性天敵草蛉 (*Mallada* spp.) 之食餌。為此，全球學者著重研究如何優化外米綴蛾飼養配方與生產流程以獲得大量的卵粒。

有害動物綜合管理 (integrated pest management; IPM) 為當今友善農業的主流思想，其中以天敵進行生物防治尤其重要 (Arun Kumar *et al*. 2018)。Hunter (2003) 認為大規模生產天敵的目標，就是用更大量、更快速及更便宜的方式，生產高質量的代用寄主，以穩定支持整個天敵供應鏈。

欲探討不同飼養配方原因，根據 Fantinou *et al*. (2008) 研究指出鱗翅目成蟲的生活史好壞主要取決於幼蟲階段積累的養分，因為成蟲能獲得更多養分的機會較少。飼養配方的差異更可影響到外米綴蛾產量上的差異，根據 Senthil Nathan *et al*. (2006) 研究指出使用高質量的飼養配方飼養外米綴蛾可得到高質量的卵粒，高質量的卵粒可生產高質量的赤眼卵寄生蜂。有鑑於此，本試驗擬探討不同飼養配方對外米綴蛾生產蛾卵的品質影響，此與 Bhandari & Regmi (2014)、Mehendale *et al*. (2014)、Rajkumari *et al*. (2014) 以及 Arun Kumar *et al*. (2018) 等使用傳統穀物作為飼料配方有所不同，本試驗添加花粉作為新的飼料配方，期能縮短外米綴蛾生活史、提升卵的品質及產卵

投稿日期：2024 年 5 月 10 日；接受日期：2024 年 7 月 23 日。
[*] 通訊作者：lihsinwu@mail.npust.edu.tw
[1] 國立屏東科技大學植物醫學系碩士生。臺灣 屏東縣。
[2] 國立屏東科技大學植物醫學系大學生。臺灣 屏東縣。
[3] 國立屏東科技大學植物醫學系副教授。臺灣 屏東縣。

量，以提升生物防治天敵其代用寄主的運用效能。

材料與方法

外米綴蛾母族群飼養

本次試驗所使用的外米綴蛾母族群，係由農業部農業試驗所姚美吉博士提供，另採自屏東地區碾米廠。飼養世代 1 年 11 世代，飼養配方參考Bernardi *et al*. (2000) 建議之配方 (米糠 94% + 砂糖 6%) 進行母族群繼代飼養。所使用的米糠購自屏東地區碾米廠，用於飼養與實驗的米糠，皆使用塑膠袋裝置於 -20℃冷凍 30 h，移除可能存活於飼料中的倉儲害蟲，並以室溫條件將米糠與乾燥劑置於 15 L 塑膠圓桶儲藏。

將混合飼料放入壓克力材質的飼育盒中，然後在飼料表面均勻灑上外米綴蛾卵粒，將絲襪套住兩側開口以防止孵化幼蟲逃逸、避免其他昆蟲入侵或成蟲羽化後逃逸，所有飼育盒均置於生長箱內。飼育盒出現成蟲後，開始捕捉置入直徑 10.5 cm 與高 14 cm 的產卵盒中，收集第 1 天所產的卵粒作為試驗材料。

不同飼養配方飼料配置

於本試驗中使用不同的飼料配方與接種相同量的外米綴蛾卵，藉以比較不同飼養配方對於外米綴蛾的效果。共有 4 種處理。

配方原料有米糠、大豆粉及花粉。每次試驗有 4 個處理配方，分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)，每個配方處理有 5 個飼養盒，每飼養盒依上述比例配置 150 g，並放置 160 粒卵粒，卵粒來源為母族群當日所產之新鮮卵粒，置於定溫 30℃與 18 L : 6 D 之生長箱中，進行 4 次重複試驗。

雌蟲百分比計算

當生長箱中的飼養盒出現成蟲，以成蟲第 1 天開始計算，每日統計雌、雄成蟲數量，共統計 21 d。將每日數據除以累積蟲數，以獲得雌蟲百分比數據。

雌成蟲重

每日統計飼養盒之成蟲性別與數量後，逢機捕捉 10 隻雌成蟲進行秤重，秤重方式參考學者 (Mehendale *et al*. 2014)，為避免活動過程中影響體重數據，將羽化之雌成蟲立即放置於 -20℃冰凍 20 min，再放入 10 隻雌成蟲於培養皿，並以微量天平進行秤重，所得數據除以 10 即可得到每隻雌成蟲平均重量。

產卵量

每日統計飼養盒內的成蟲性別與數量，逢機選擇 5 對雌雄成蟲，將之置於產卵盒，產卵盒底部放置 1 培養皿 (10 mL) 以收集雌蟲所產卵粒，每天提供含 10% 蜂蜜水衛生紙片 (2 × 2 cm)，並統計每日產卵量，共計 5 d。

百卵重

於當天從飼養盒隨機選 5 對雌雄成蟲放入產卵盒，提供 10% 蜂蜜水衛生紙片，讓成蟲於產卵盒進行交配 24 h，隔天統計產卵量，並且逢機挑選當日產下之卵粒 100 粒於培養皿，再將培養皿秤重，即可得到百卵重數據。為了減少水分與其他變因的影響，百卵重的計量僅取交配第 1 天之成蟲所產下之卵粒。

每毫升卵生產成本計算

市售外米綴蛾卵粒以 1 mL 做為市售單位，而 1 mL 卵粒約有 16,000 粒卵。將本試驗的 4 種處理所花費的成本經過計算後得到總費用，再除以 4 種處理個別平均卵數，即可得到單粒卵的成本。得到單粒卵成本後再乘以 16,000，可推估 1 mL 卵粒成本。

數據統計分析

本次試驗所使用 R 統計分析軟體進行分析，先以單因子變異數分析 (analysis of variance; ANOVA) 來比較試驗結果，若具有顯著差異 ($P < 0.05$)，以 Fisher’s Least Significant Difference test (LSD) 比較處理間差異的顯著性。

結果

雌成蟲累積羽化率

自成蟲出現第 1 天開始計算，每天統計雌

雄數，共 21 d。得到雌成蟲之累積羽化率 (圖 1)。雌成蟲累積出現率的折線圖中，花粉配方比其他 3 個配方更早達到 50% 的雌成蟲累積羽化率。對照天數，C 配方第 10 天達到 50% 的雌成蟲累積羽化率，A 配方第 15 天、B 配方第 15.5 天及 D 配方第 14 天，相比添加 10% 花粉的 C 配方，其他處理皆延遲了 4−5 d。

雌成蟲生活史差異

自成蟲出現第 1 天開始計算，共記錄 21 d，雌蟲百分比的計算方式為 (雌蟲數/總重數) × 100%。發現雌成蟲百分比顯示，有加入花粉處理的 C、D 配方 (47.03%、45.75%) 顯著高於米糠 90% + 大豆粉 10% 的 B 配方 (42.86%) (圖 2，$F_{3,10} = 4.80$，$P = 0.02 \times 10^{-6}$)。而 100% 米糠的 A 配方 (44.56%) 則與其他處理皆無顯著差異。

雌成蟲從數據中顯示，花粉 10% 的 C 配方 (0.26 g) 顯著差異高於其他 3 個配方 (A、D 為 0.22 g，B 為 0.20 g)，由此可得知 10%

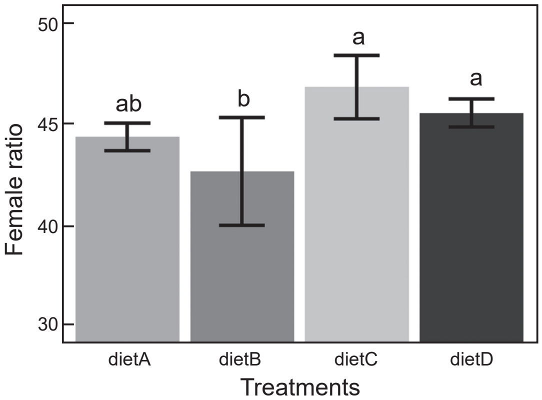

圖 2. 外米綴蛾飼養於 4 種不同比例配方雌蟲百分比。4 個處理分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)。雌蟲百分比的計算方式為：(雌蟲數/總重數) × 100%。$F_{3,10} = 4.80$，$P = 2.00 \times 10^{-8}$。

Fig. 2. Percentage of *Corcyra cephalonica* females under four different proportional formulas for rearing. A is 100% rice bran, B is 90% rice bran +10% soybean powder, C is 90% rice bran + 10% pollen, and D is 90% rice bran + 5% soybean powder + 5% pollen.

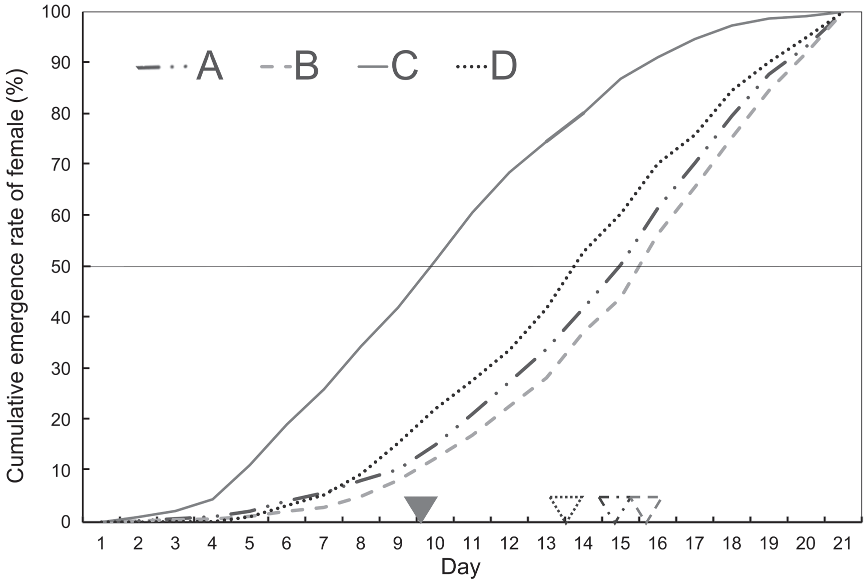

圖 1. 外米綴蛾飼養在 4 種不同比例配方，統計 21 d 雌成蟲累積出現率與 50% 的雌成蟲累積羽化率。4 個處理分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)。倒三角形為 50% 的雌成蟲累積羽化率。

Fig. 1. The *Corcyra cephalonica* was reared on four proportional formulas and the cumulative emergence rate of female adults over 21 d and the 50% emergence rate. A is 100% rice bran, B is 90% rice bran +10% soybean powder, C is 90% rice bran + 10% pollen, and D is 90% rice bran + 5% soybean powder + 5% pollen. The inverted triangle represent 50% emergence rates.

花粉含量可增加雌成蟲的體重 (圖 3，$F_{3,44}$ = 14.30，P = 1.20 × 10^{-6})。

總產卵量的計量上，花粉 10% 的 C 配方 (平均每天產下 785.95 粒) 顯著差異高於其他 3 個配方 (A、B 及 D 分別為 555.8 粒、641.05 粒及 662.65 粒)，由此可得知 10% 花粉配方所飼養出的雌蛾亦可有較高的產卵量 (圖 4，$F_{3,76}$ = 13.41，P = 4.10 × 10^{-6})。

百卵重數據顯示，花粉 10% 的 C 配方 (4.61 mg) 同樣顯著差異高於其他 3 個飼養配方 (A、B 及 D 分別為 4.03 mg、3.73 mg 及 4.03 mg)，由此可以得知花粉配方所飼養的雌蛾，所產的卵粒重量亦顯著的提升 (圖 5，$F_{3,76}$ = 18.95，P = 2.80 × 10^{-9})。

每毫升卵粒生產成本

探討不同配方的同時，也需考量其生產成本，以利未來生物防治施行能有規模且制式化的生產流程。而探討此次試驗的成本，同時也一併將市售台灣糖業股份有限公司 (以下簡稱

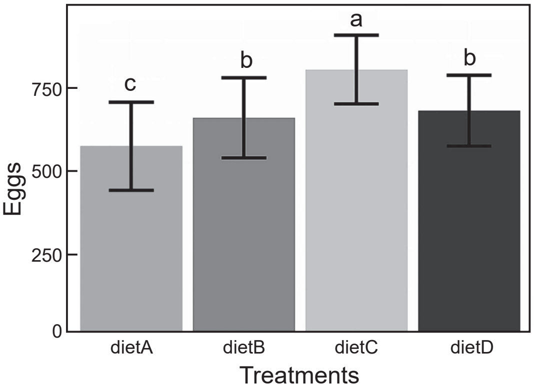

圖 4. 比較 5 對外米綴蛾成蟲在 4 種不同比例配方飼養下 5 d 產卵總量差異。4 個處理分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)。$F_{3,76}$ = 13.41，P = 4.10 × 10^{-6}，Analysis of Variance (ANOVA) test。

Fig. 4. Comparing the total fecundity over five days for five pairs of adult *Corcyra cephalonica* reared under four different proportional formulas. A is 100% rice bran, B is 90% rice bran + 10% soybean powder, C is 90% rice bran + 10% pollen, and D is 90% rice bran + 5% soybean powder + 5% pollen.

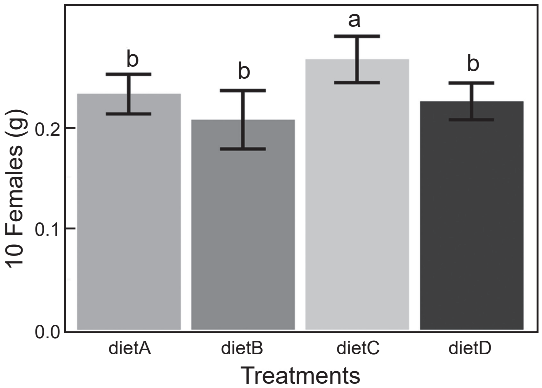

圖 3. 在 4 種不同比例配方下飼養 10 隻外米綴蛾雌成蟲重。4 個處理分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)。$F_{3,44}$ = 14.30，P =1.20 × 10^{-6}，Analysis of Variance (ANOVA) test。

Fig. 3. The weight of 10 female *Corcyra cephalonica* adults reared under four different proportional formulas. A is 100% rice bran, B is 90% rice bran +10% soybean powder, C is 90% rice bran + 10% pollen, and D is 90% rice bran +5% soybean powder +5% pollen. $F_{3,44}$ = 14.30, P = 1.20 × 10^{-6}, Analysis of Variance (ANOVA) test.

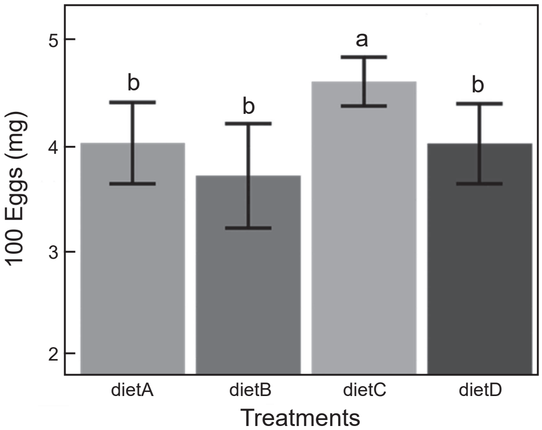

圖 5. 在 4 種不同比例配方飼養下外米綴蛾百卵重。4 個處理分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)。$F_{3,76}$ = 18.95，P = 2.80 × 10^{-9}，Analysis of Variance (ANOVA) test。

Fig. 5. The egg mass weight of *Corcyra cephalonica* reared under four different proportional formulas (mg): A is 100% rice bran, B is 90% rice bran +10% soybean powder, C is 90% rice bran + 10% pollen, and D is 90% rice bran +5% soybean powder + 5% pollen.

台糖) 販售之價格一起探討。(總花費/平均卵數) × 16,000，即可得到 1 mL 外米綴蛾卵粒成本，並將各成本做成長條圖進行比較 (圖 6)。A 為 100% 米糠配方，所使用之米糠為 150 g，共花費 1.25 元，5 d 之產卵數為 555.8 粒，換算生產每 1 mL 卵粒需要花費 35.98 元；B 配方為 135 g 米糠 + 15 g 大豆粉，共計 4.125 元，5 d 之產卵量為 641.05 粒，換算每 1 mL 合計花費 102.96 元；C 配方為 135 g 米糠 + 15 g 花粉，共計 13.625 元，5 d 之產卵量為 785.95 粒，換算每 1 mL 所需花費成本為 145.05 元；D 配方為 135 g 米糠 + 7.5 g 大豆粉 + 7.5 g 花粉，共計 8.875 元，5 d 之產卵量為 662.65 粒，換算每 1 mL 成本為 99.6 元。

討論

根據文獻資料顯示，花粉的營養品質對各種昆蟲種類的發育與繁殖均有顯著影響，不同花粉類型間，蛋白質與脂肪含量間變化多樣 (Vaudo *et al*. 2016)。二星瓢蟲 (*Adalia bipunctata*) 的發育與繁殖，即受到所提供食物類型所影響，當幼蟲以粉蝨的蛹為食，能夠最快發育至成蟲並有最重的體重 (De Clercq *et al*. 2005)。

研究顯示以基因轉殖的玉米花粉為食物，可能降低斑點瓢蟲 (*Coleomegilla maculata*) 的蛻皮與羽化成功率 (Duan *et al*. 2002)。這顯示花粉的來源與成分，對昆蟲發育與存活具重大影響。Kast *et al*. (2019) 的研究亦強調花粉的營養價值，其中含有大量維生素、礦物質及生物活性化合物，對於飲食補充與各種生物活性都是必需的。同時亦有研究證實，花粉可作為暗黑長脊盲蝽 (*Macrolophus pygmaeus*) 的替代、補充食物，增進蛻皮為成蟲的比率，以及成蟲體重與卵細胞數量 (Vandekerkhove & De Clercq 2010)。這些研究結果顯示花粉的營養價值，對昆蟲種類發育與繁殖具有顯著助益，亦凸顯大量飼育天敵昆蟲時，將花粉作為添加物的重要性。

在 4 種不同配方的處理中，花粉配方在雌蟲累積羽化率，較其他 3 個配方更有效率，可使有效縮短飼養週期。4 種不同配方的處理中，花粉配方在雌成蟲體重、產卵量及百卵重等方面，皆優於其他 3 個配方。另外米糠 100% 的處理對雖不影響雌成蟲的體重，卻顯著降低雌成蟲的總產卵量，但所產下的卵粒則比 B 配方 (米糠 90% + 大豆粉 10%) 更重。由此可推斷大豆粉 10% 的添加能夠刺激雌成蟲產下較多的卵，但是卵的品質卻同時降低，因此大豆粉不適合作為外米綴蛾的飼養配方 (圖 2、圖 3 及圖 4)。

有關不同配方所生產的外米綴蛾卵粒成本，本試驗的花粉配方雖較其他處理與台糖販賣的價格高，但花粉配方所獲之卵粒品質卻是最優異的。將本次試驗中最優異之花粉配方與其他研究之最優異配方予以比較 (表 1)，發現花粉配方較其他配方之發育期縮短 9.6–22.82 d；百卵重方面，本試驗最優異配方為 4.6 mg，與直接添加維生素 E 的處理相近 (4.8 mg)，而維生素即為花粉中相當重要的成分 (Begum & Qamar 2015)，代表花粉具有成為未來飼養外米綴蛾之新添加物的潛力，至於

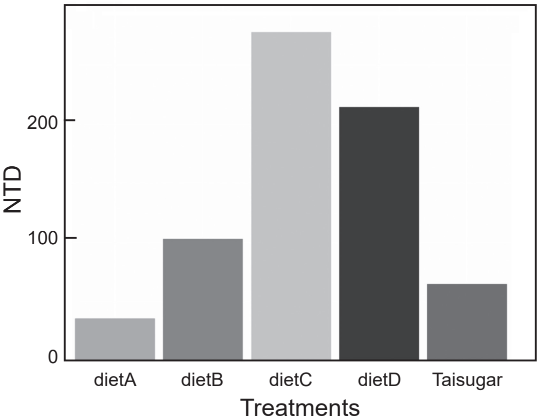

圖 6. 不同配方每毫升卵的成本：(總花費/平均卵數) × 16,000。4 個處理分別為 A 配方 (米糠 100%)、B 配方 (米糠 90% + 大豆粉 10%)、C 配方 (米糠 90% + 花粉 10%) 及 D 配方 (米糠 90% + 大豆粉 5% + 花粉 5%)。Taisugar 為台灣糖業股份有限公司市售外米綴蛾購買價格。

Fig. 6. The cost per 1 mL of eggs for each feed formula. A is 100% rice bran, B is 90% rice bran + 10% soybean powder, C is 90% rice bran + 10% pollen, and D is 90% rice bran + 5% soybean powder + 5% pollen. Taisugar represents the price of a commercial product in Taiwan.

表 1. 將本試驗最優異之花粉配方與其他學者研究中最優異的外米綴蛾飼料配方相比較，比較的重點有發育期、百卵重及產卵量 3 個層面。

Table 1. Comparing the optimal pollen formula used in this experiment with the optimal *Corcyra cephalonica* feed formula from other scholars regarding development period, egg mass weight, and egg production.

Diets	Developmental period	Weight of 100 eggs (mg)	Fecundity	References
Sorghum : Mille : Maize : Groundnut (3 : 3 : 3 : 1)	37.60	4.20	39.700	(Begum & Qamar 2015)
99.5% (Sorghum : Millet : Maize : Groundnut (3 : 3 : 3 : 10)) + 0.5%Vit. E	38.80	4.80	39.900	(Begum & Qamar 2015)
Sorghum 100%	50.82	4.26	-	(Pathak *et al*. 2010)
Sorghum 70% + Gram 30% + 5 g Powdered yeast	-	3.73	98.856	(Mehendale *et al*. 2014)
Rice 125 g + wheat 125 g + Groundnut 25 g	42.55	-	166.625	(Rajkumari *et al*. 2014)
Sorghum 95% + Groundnut 5%	47.33	-	312.330	(Arun Kumar *et al*. 2018)
Rice bran 90% + pollen 10%	28.00	4.60	157.190	

對成本的控管與降低，則有賴後續持續優化最佳添加比率與對繁殖功能影響的詳細試驗。

致謝

本研究感謝科技部計畫 MOST-111-2313-B-020-003-MY3 與農業部防檢署 112AS-5.5.3 -BQ-B1 之經費支持。同時由衷的感謝各位編輯與兩位匿名審查委員不吝撥冗斧正，謹致由衷謝忱。

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Influence of Pollen Provisioning on Fecundity and Life History Traits of the Rice Moth, *Corcyra cephalonica* (Lepidoptera: Pyralidae)

Jia-Hong Wu[1], Cheng-Jin Lai[2], Xin-Ci Hong[1], and Li-Hsin Wu[3,*]

Abstract

Wu, J. H., C. J. Lai, X. C. Hong, and L. H. Wu. 2024. Influence of pollen provisioning on fecundity and life history traits of the rice moth, *Corcyra cephalonica* (Lepidoptera: Pyralidae). J. Taiwan Agric. Res. 74(3):251–258.

Biological control is an essential method in integrated pest management. Although *Corcyra cephalonica* (Stainton) is a vital storage pest, its fresh eggs can be the most effective alternative host for various important natural enemies and parasitoid wasps. In this experiment, rice bran, soybean powder, and pollen were mixed with four different proportions according to Literature and feeding experience and measured (1) the cumulative occurrence rate of female adults, (2) percentage of females, (3) female weight, (4) the fecundity, (5) 100 eggs weight, and (6) the production cost per milliliter of the egg is expected to improve the quality of fecundity. The results showed that the adult females reared with 10% pollen could shorten the life cycle and early eclosion and have better growth parameters than other treatments. Therefore, pollen was of great nutritional value for the mass-rearing external rice moths. However, compared with the production cost per milliliter of eggs, the cost of adding pollen treatment is higher than that of the other three formulas. Still, the fecundity of each female moth is as high as 157.19 ± 12, which is quite close to the best fecundity record of 166.63 in the literature. Pollen is rich in a variety of vitamins. Further fine-tuning and experiments are needed to determine the effective and economical addition of pollen to the moth's eggs. The adequate minimum proportion of pollen should be determined as well. As such, biological control materials with high quality, yield, and more economical cost can be applied to automatic production and optimize the whole production process of parasitoid wasp biological control.

Keywords: Food formula, Pollen, *Corcyra cephalonica*, Fecundity, Life-cycle assessments.

Received: May 10, 2024; Accepted: July 23, 2024.

[*] Corresponding author, e-mail: lihsinwu@mail.npust.edu.tw

[1] Master Students, Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung County, Taiwan.

[2] Undergraduate Student, Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung County, Taiwan.

[3] Associate Professor, Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung County, Taiwan.

台灣農業研究 (J. Taiwan Agric. Res.) 73(4):259–268 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0004

研究報告

運用自動微噴霧設施降溫提升「玉荷包」荔枝開花率與防治荔枝細蛾

徐智政 [1]　陳薪嘵 [1]　方信秀 [2,*]

摘要

徐智政、陳薪嘵、方信秀。2024。運用自動微噴霧設施降溫提升「玉荷包」荔枝開花率與防治荔枝細蛾。台灣農業研究 73(4):259–268。

為提升「玉荷包」荔枝開花率，本研究嘗試以自動噴霧設施於 12 月定時噴霧降溫，再於 3 月調查開花比例。結果顯示，4 種不同時間噴霧處理的開花率分別為不噴霧降溫 18.3%、白天夜晚噴霧 32.1%、白天噴霧 42.9% 及夜間噴霧 26.7%，白天噴霧為提升荔枝開花率較佳的時段。白天噴霧的 2 種處理開花率皆顯著較不噴霧對照組高，而夜間噴霧處理並無顯著增加開花率。進一步使用同套噴霧系統測試以農藥噴霧防治荔枝細蛾的效果，結果顯示 4 種防治處理的為害率分別為：無用藥對照 61%、自動噴藥 10%、人工噴藥 0% 及夜間綠光 light-emitting diode (LED) 燈照 2%。人工噴藥與夜間綠燈防治皆有極佳的防治效果，農藥自動噴霧方式雖可節省人力且降低荔枝細蛾為害率，但為害率仍有改進空間。

關鍵詞：荔枝、開花率、荔枝細蛾、溫度、農藥。

前言

荔枝 (litchi, *Litchi chinensis* Sonn.) 屬無患子科 (Sapindaceae) 的亞熱帶與熱帶常綠果樹，僅南北迴歸線一帶可順利生產，中國、泰國、印度、臺灣、越南及澳洲為主要生產國 (Mitra & Pathak 2010)，相當值得開發與研究。

臺灣主要荔枝栽培品種依產期早晚分為「玉荷包」、「黑葉」、「竹葉黑」、「糯米糍」及「桂味荔枝」。種植地區集中於臺灣中南部，其中以高雄市 3,179 ha、臺中市 1,873 ha 及南投縣 1,331 ha 為主。荔枝於 2022 年總種植面積為 9,640 ha，相較 2012 年的 11,638 ha，於 10 年內快速減少近 2,000 ha，約減少 17.1% 的面積 (農糧署農情報告資源網，https://agr.afa.gov.tw/afa/afa_frame.jsp)，因農民年齡老化與極端天氣趨於常態造成生產不穩定，進而使栽培面積逐漸減少 (Chang *et al*. 2017)。

「玉荷包」荔枝為早熟種，較「黑葉」荔枝與「糯米糍」荔枝較易達到花芽分化之低溫需求 (Chang 1999)。但近年暖冬發生率較高 (Fang *et al*. 2022a)，造成開花著果穩定性降低，影響收益。許多研究探討如何穩定荔枝生產，包括疏梢培養健壯結果枝、疏花減少養分消耗、環刻養分蓄積及適當的水分管理 (Yuan & Huang 1993; Hieke *et al*. 2002; Huang *et al*. 2003; Olesen *et al*. 2013)。荔枝入秋後於適度低溫與乾旱刺激下，有助荔枝營養生長轉換為生殖生長，花芽分化受到溫度、水分逆境及枝條成熟度所影響 (O'Hare 2002; Malhotra *et al*. 2018; Su *et al*. 2021)。溫度可決定新芽發展為葉片或是花朵 (Menzel *et al*. 1989)。必須有一段足夠的低溫期才可誘導花芽分化 (Chen & Huang 2005; Chen *et al*. 2013)。冬季低溫誘導荔枝花芽分化成花芽混合葉芽基體 (rudimentary leaves) 與花芽基體 (panicle primordia)，

投稿日期：2024 年 4 月 19 日；接受日期：2024 年 7 月 31 日。
* 通訊作者：show@tari.gov.tw
1 農業部農業試驗所鳳山熱帶園藝試驗分所熱帶果樹系助理研究員。臺灣 高雄市。
2 農業部農業試驗所鳳山熱帶園藝試驗分所熱帶果樹系副研究員。臺灣 高雄市。

若持續處於足夠低的溫度，葉芽基體將停止生長，花芽基體則持續發展為完整的圓錐花序。暖冬或暖春可使葉芽基體持續生長為完整葉片，而花芽基體則停止生長或萎縮，也可能形成帶葉花序，影響開花率 (Yang *et al*. 2017)。由於全球暖化，暖冬或是溫暖的春天會抑制花芽分化或花芽敗育而影響荔枝產量 (Liu *et al*. 2019)。本研究透過白天與夜晚噴霧降溫的概念，探討不同時間噴霧降溫增加開花穩定度的效果。過去研究指出，「玉荷包」荔枝產量與前一年 12 月的氣溫相關性最高 (Fang *et al*. 2022a)，故本試驗於 12 月進行噴霧降溫試驗。

除了使用噴霧降溫的試驗，進一步使用此套噴霧設施於夜間噴藥進行蟲害防治效果評估，主要觀察的防治對象為荔枝細蛾。荔枝細蛾 (*Conopomorpha sinensis* Bradley, litchi fruit borer) 為影響荔枝產業最嚴重的害蟲，易導致嚴重減產 (Chen *et al*. 2022)。以人工噴藥與不噴藥做為對照。因荔枝細蛾對光十分敏感，主要於夜間活動，白天則趨於靜止狀態。研究結果證實夜間使用 light-emitting diode (LED) 綠光照射，可同時具有高效率的防治效果與良好的果實品質 (Fang *et al*. 2022b, 2023)，因此將夜間綠光 LED 燈照防治方式也納入，共同比較不同防治方式之防治效率，作為未來產業運用之參考。

材料與方法

本試驗地點位於高雄市旗山區許氏農民「玉荷包」荔枝果園 (經緯度座標：22°46'05.3"N、120°26'25.0"E)，全園進行相同條件的灌水、施肥及病蟲害田間管理。微霧噴頭內含濾心操作壓力 200 pound per square inch (PSI)，噴霧角度 75°– 80° (AQUA AIRCON C2, Shang-I, Taichung, Taiwan)，水源使用動力噴霧機 (W-45B, WULI, Taichung, Taiwan) 加壓，並設定 4 個迴路，分別設有電磁閥作為定時開關使用。以硬質水管連接至各區後，再使用軟管分接，每株植株設置 7 個微霧噴頭，包括離地上 3.5 m 處設置 5 個噴頭環繞植株，另有 2 個噴頭以軟管延長至植株內部離地面 1.5 m 處。

噴霧降溫試驗

噴霧降溫處理時間自 2021 年 12 月 1 日開始至 12 月 31 日，溫度紀錄器 (HOBO Pro v2, Onset, Boston, MA, USA) 每 5 min 記錄 1 筆資料，連續記錄 1 mo，每個處理設置 1 個紀錄器設置於玉荷包植株下方，離地面 1 m，陽光全日不會照射的區域，噴霧水來源水塔使用冰水機控制於 10℃。4 種噴霧時間處理分別為 (A) 不噴霧對照組、(B) 白天噴霧組 10:00–16:00、(C) 夜間噴霧組 01:00–07:00 及 (D) 白天夜晚噴霧組。各處理時間中每分鐘噴霧 36 mL，每噴霧 1 min 停止 30 s，每組各 24 株「玉荷包」，株齡 15 年，株高約 2.5 m 高。2022 年 3 月 15 日計算開花率，每株開花率調查方式為植株東西南北與頂部各隨機調查 2 穗，調查每穗是否有花芽抽出，無論是帶葉花序或純花序皆視為花芽有順利分化，皆標註為有花芽抽出，每株共調查 10 穗計算該株開花率。之後再將各處理的開花率平均，取得不同處理的平均開花率，另進行不同噴霧處理的不同開花率統計比較。

2019 年 12 月全天氣象變化資料，取自中央氣象署網站 (Central Weather Administration) 高雄溪埔站 (代號 C0V350)，海拔高度 36 m，(經緯度座標：22°44'18.9"N、120°26'48.5"E)，此氣象站距離試驗地點直線距離約 3.01 km。據 2019–2023 年的 12 月逐小時氣象資料顯示，全日氣溫於 7:00 之後開始增加，直至 14:00 達到全日最高溫，之後再逐漸減少，故本試驗將噴霧時間安排於夜間 1:00−7:00，與白天 10:00−16:00 兩個時段 (圖 1)。

自動微霧噴藥試驗

防治荔枝細蛾的試驗處理包括 (A) 不噴藥對照組、(B) 夜間自動噴霧組、(C) 白天人工噴藥組及 (D) 夜間綠光 LED 燈照組。藥劑種類與濃度分別為亞滅培水溶性粉劑 (active ingredient (a.i.) acetamiprid, 20% soluble powder)、丁基加保扶可濕性粉劑 (a.i. carbosulfan, 40% wettable powder)、加保利可濕性粉劑 (a.i. carbaryl, 50% wettable powder)、撲滅松可濕性粉劑 (a.i. fenitrothion, 40% wettable powder)、

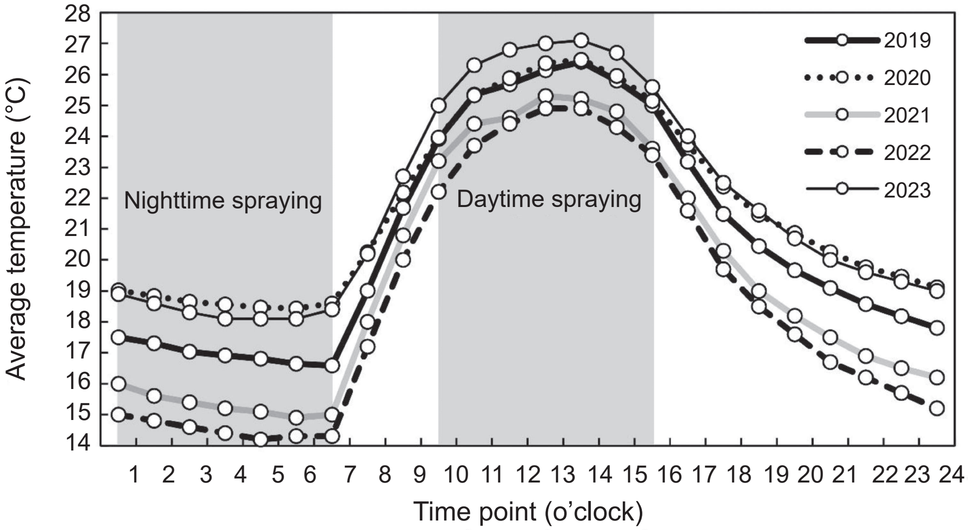

圖 1. 高雄溪埔氣象站 12 月全日平均溫度與本試驗噴霧時段。

Fig. 1. Daily average temperature of Kaohsiung Xipu Meteorological Station in December and spraying periods in this experiment.

芬殺松乳劑 (a.i. fenthion, 50% emulsifiable concentrate) 及賽洛寧微乳劑 (a.i. lambda-cyhalothrin, 2.5% microemulsion)。施用時間為 2022 年，施藥日期與藥劑種類如附錄所示。自動噴霧噴藥時間為夜晚 20:00 開始。夜間 LED 綠燈 (12 W LED lamp, Innovta, Kaohsiung, Taiwan) 架設於株與株之間離地 3.5 m 處，燈泡設置密度長 4 m 寬 6 m，於 3 月 28 日開始燈照，燈照時間為每日 18:00−06:00。使用燈具 12 W，電壓為 110 V，光譜範圍於 500–560 nm 之間，發光效率為 (113 lm W^{-1})。

所有處理於 6 月 5 日採收調查果實為害率。為害率調查方式為每處理內植株逢機取 10 株，每株逢機取 10 粒荔枝果實，每處理共採樣 100 粒果實。每粒果實皆使用刀具縱向開後，觀察果實種子切面與果蒂內部是否有荔枝細蛾為害。

資料於 Microsoft Office Word 與 Excel 進行資料輸入與圖表繪製，使用 SAS Enterprise Guide 7.1 版本進行統計分析，先進行變方分析確定處理因子具顯著差異水準後，再進行最小顯著性多重比較 (least significant difference; LSD) ($P < 0.05$)。

結果

噴霧降溫提升開花率試驗

噴霧降溫試驗的 4 種處理分為不噴霧對照組、白天噴霧組、夜間噴霧組及白天夜晚噴霧組等 4 種處理。試驗結果顯示，全日氣溫大多介於 15–30℃之間，而夜晚溼度大多超過 90%。本試驗結果顯示，4 種噴霧降溫處理沒有造成明顯的處理植株周遭環境的溫度與溼度大幅變化 (圖 2)。

進一步觀察白天與夜晚噴霧時段之溫溼度資料，4 種處理之白天噴霧時段平均溫度為 24.7–25.5℃，夜晚噴霧時段平均溫度為 16.2–16.4℃。白天噴霧時段平均溼度為 65.4–68.9%，夜晚噴霧時段平均溼度為 94.3–95.9%。

以不噴霧對照組為比對基準，白天噴霧時段的白天噴霧組與白天夜晚噴霧組的平均溫度下降 0.8℃與 0.4℃，平均溼度下降 0.4% 與 1.8%；而夜晚噴霧時段之夜間噴霧組與白天夜晚噴霧組的平均溫度增加 0.1℃與 0℃，平均溼度增加 0.7% 與 0.6% (表 1、表 2)。

四種不同噴霧處理分別調查24株「玉荷包」荔枝，不同開花率之株數統計於圖 3，不噴霧對照組有 7 株開花率為 0%，為 4 種處理中最

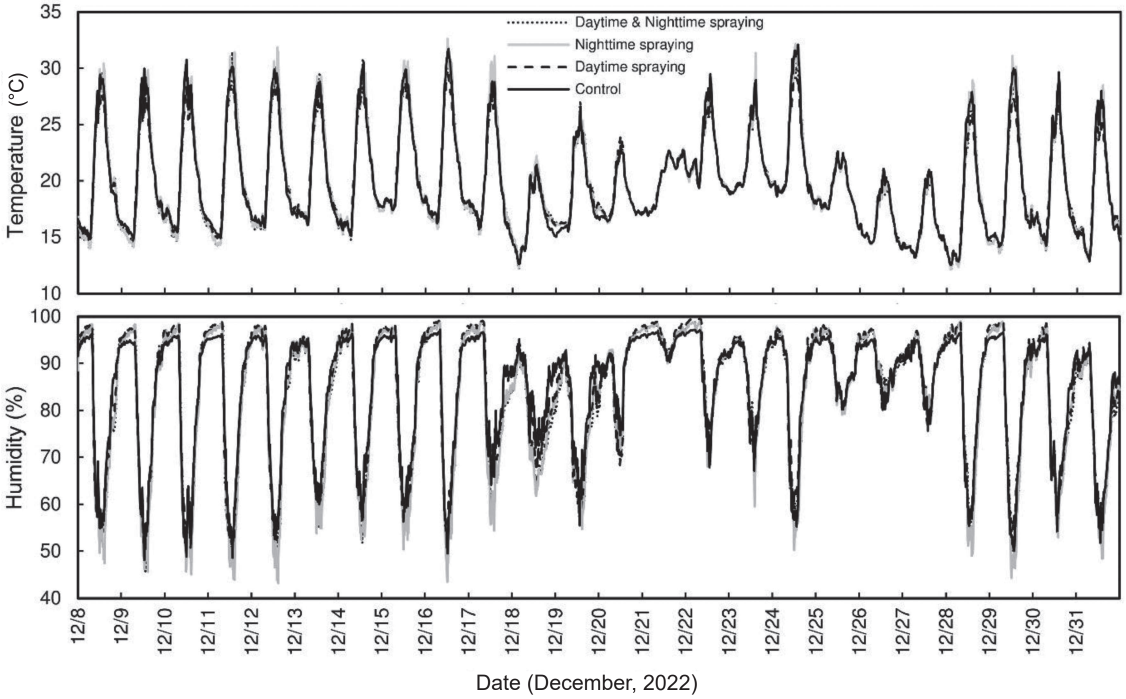

圖 2. 4 種不同噴霧處理之每日溫溼度變化。

Fig. 2. Daily temperature and humidity changes of 4 different spraying treatments.

表 1. 4 種不同噴霧處理之於白天噴霧時段與夜晚噴霧時段之平均溫度。

Table 1. Average temperature of 4 different spraying treatments applied during day and nighttime spraying periods.

	Average temperature (℃) at different treatments			
Period	Control	Nighttime spraying	Daytime spraying	Daytime & Nighttime spraying
10:00−16:00	25.5	25.5	24.7	25.1
01:00−07:00	16.3	16.2	16.4	16.2
All day	19.8	19.9	19.7	19.7

表 2. 4 種不同噴霧處理之於白天噴霧時段與夜晚噴霧時段之平均溼度。

Table 2. Average humidity of 4 different spraying treatments applied during day and nighttime spraying periods.

	Average humidity (%) at different treatments			
Period	Control	Nighttime spraying	Daytime spraying	Daytime & Nighttime spraying
10:00−16:00	68.9	65.4	68.5	67.1
01:00−07:00	94.3	95.0	95.9	94.9
All day	84.7	83.3	84.9	83.6

多植株者。而白天噴霧組與白天夜晚噴霧組全部植株都有不同比例的開花。將所有的開花率資料進行統計分析，不噴霧對照組、夜晚噴霧組、白天噴霧組及白天與夜晚噴霧組等 4 種處理的開花率分別為 18.3%、26.7%、42.9% 及 32.1%。其中白天噴霧組、白天夜晚噴霧組及

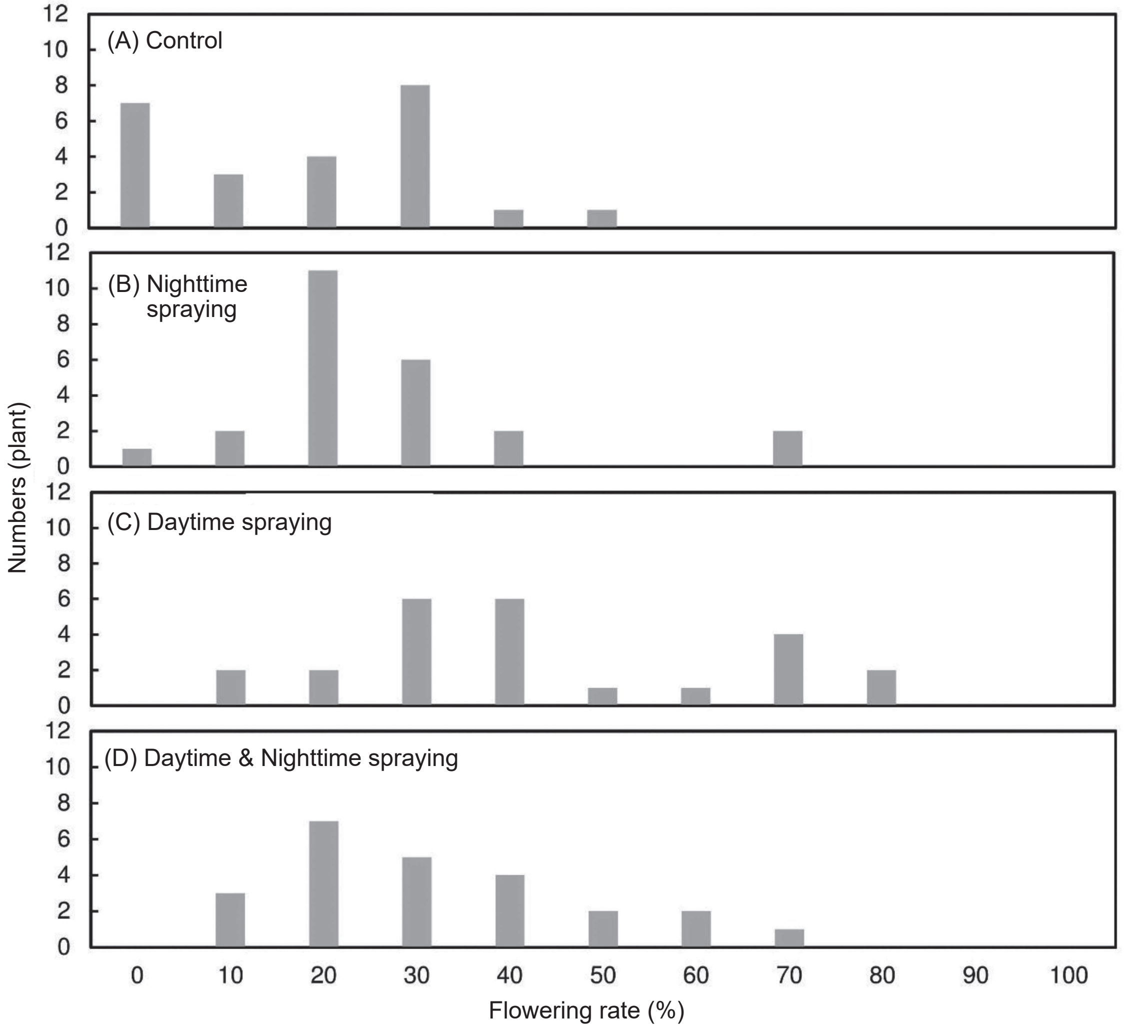

圖 3.　「玉荷包」荔枝進行 4 種不同噴霧處理的不同開花率株數統計。

Fig. 3.　Statistical analysis of the numbers of ‘Yu-Her-Pao’ litchi plants with different flowering rates after 4 different spraying treatments.

不噴霧對照組的平均開花率皆有達顯著性差異，而夜晚噴霧組並沒有與不噴霧對照組的平均開花率達到顯著性差異 (表 3)。

夜晚自動噴藥防治荔枝細蛾試驗

防治荔枝細蛾試驗包括不噴藥對照組、夜晚自動噴藥組、白天人工噴藥組及夜間綠光 LED 燈照組等 4 種處理。於果實成熟後調查為害率分別為 61%、10%、0% 及 2%。以人工噴藥組與夜間綠光 LED 燈照組的為害率控制

表 3.　「玉荷包」荔枝進行 4 種不同噴霧處理之平均開花率。

Table 3.　Average flowering rate of ‘Yu-Her-Pao’ litchi under 4 different spraying treatments.

Treatment	Flowering rate
Control	18.3 ± 3.04 c[z]
Nighttime spraying	26.7 ± 3.27 bc
Daytime spraying	42.9 ± 4.31 a
Daytime & Nighttime spraying	32.1 ± 3.40 b

[z] Mean ± standard error. Means denoted with the same letter are not significantly different each other at level $P = 0.05$ of least significant difference (LSD) test ($n = 24$).

表 4. 4 種不同防治方式之荔枝細蛾為害率。
Table 4. Damage rates of litchi fruit borer under 4 different litchi fruit borer control methods.

Treatment	Litchi fruit borer damage rate (%)
Control (none pest control)	61.0 ± 1.4 a[z]
Automatic pesticide spraying	10.0 ± 3.0 b
Manual pesticide spraying	0 ± 0 c
Green light-emitting diode (LED) light for nighttime illumination	2.0 ± 1.4 bc

[z] Mean ± standard error. Means denoted with the same letter are not significantly different each other at level $P = 0.05$ of least significant difference (LSD) test ($n = 100$).

顯著較佳，夜晚自動噴藥組亦顯著優於不噴藥對照組。

討論

荔枝於冬季有足夠的低溫期開花率普遍較穩定，抽花穗期較低的溫度花芽分化較穩定，而開花期較高的溫度可增加授粉昆蟲活性且光合效率較高，使產量較高，不同的荔枝品種對溫度變化的敏感度不同 (Qi & Ouyang 2017; Qi & Ou 2019)。高溫環境影響形態發育、生理及化學物質改變，而導致產量與經濟的損失 (Sharma & Manjeet 2020)。為使作物可順利生產，於溫室內搭配噴霧、風扇或空調，以增加溼度與蒸散作用的方式達到降溫目標，於溫室內可讓白天溼度由 40–60%，提升至 70–90%，並使白天平均溫度降低 2–5℃ (Eduard *et al*. 2022)。自動噴霧降溫設施目前已嘗試於菠菜與葡萄等生產使用運用。以較喜好生長於冷涼氣候的菠菜為例，溫室內每日最高溫平均為 35.1℃，噴霧處理的每日最高溫平均降為 33.9℃，全日的平均溫度降低 0.4℃，全日平均溼度增加 5%，最終產量可增加 30% (Tai *et al*. 2020)。溫室的高溫會影響葡萄開花期的光合作用與產量減少，在 1、2 及 3 h 的噴霧，空氣溼度可由 40% 提升至 90%，分別可以降溫 5.12℃、5.09℃及 5.17℃，噴霧環境可增加葉綠素含量與光合作用效率 (Zheng *et al*. 2021)。

本試驗結果顯示，白天噴霧並未大幅提升環境的溼度與降低環境溫度，以不噴霧對照組為基準，兩個有進行白天噴霧的處理，平均溫度分別下降 0.8℃與 0.4℃ (露地栽培屬於開放空間，所以降溫幅度有限，但是卻可維持周遭環境於一相對較低的氣溫)。溼度部分，以全天溼度於 50–100% 之間變化來說，白天噴霧時的平均溼度卻反而分別下降 0.4% 與 1.8%，表示白天以露天噴霧的方式並沒有辦法大幅提升溼度，進而達到促進蒸散降溫的作用；而對照組的夜晚溼度高達 94.3%，兩個進行夜間噴霧處理的濕度分別只有提升 0.7% 與 0.6%，而平均溫度則幾乎沒有變化。此結果顯示，因噴霧處『露天開放』環境，面積達 0.5 ha，不易大幅提升白天溼度，也因此無法有效降溫，而夜間噴霧時段平均溼度高達 94.3%，可提升溼度的空間很少，即使用冰水機送出 10℃冰水噴霧降溫，亦無法達到大幅降溫的效果。但是，相對氣溫較低的周遭環境與較高的溼度，將可減少白天的維持呼吸消耗與對花穗的高溫傷害，有助於花穗開花。

比較不同噴霧降溫處理的開花率結果顯示，每處理調查 24 株，無噴霧對照組開花率 18.3%，夜晚噴霧組開花率 26.7%，兩者間沒有達顯著性差異，而白天噴霧組與白天夜晚噴霧組的開花率為 42.9% 與 32.1%，與無噴霧對照組比較皆有達顯著性差異。進一步觀察 4 種噴霧處理之不同開花率株數統計，可發現不噴霧對照組完全沒開花比例最高，白天噴霧組與白天夜晚噴霧組之所有植株則皆有不同比例的開花率，可見白天噴霧對於開花率提升有部分幫助。

對農民生產而言，開花率愈高預期產量愈高。一般而言，期望「玉荷包」荔枝達到 8 成以上的開花率，才有較穩定的產量。於本試驗結果中，白天噴霧組與白天夜晚噴霧組之 10:00–6:00 溫度較對照組低 0.8℃與 0.4℃，結

果顯示白天噴霧顯著提升開花率 1–2 成，而夜晚噴霧並沒有顯著提升開花率。雖然使用白天噴霧方式可部分提升開花率，但對於達到全區 8 成以上開花率的栽培技術仍待持續開發。

使用同套噴霧系統，進一步探討自動噴藥對荔枝細蛾的為害率控制能力，因荔枝細蛾主要活動時間為夜晚 (Fang *et al*. 2022b, 2023)，嘗試於夜晚自動噴霧噴藥以增加防治效率。試驗結果顯示，不施藥對照組果實為害率為 61%，因果實成熟才調查果實為害率，早期為害的果實大多已落果，所以實際的為害率應比 61% 還高上許多。自動噴藥、白天人工噴藥及夜間綠光 LED 燈照的為害率分別為 10%、0% 及 2%，皆與不施藥對照達顯著性差異。其中，人工噴藥與夜間綠光 LED 燈照防治效果皆相當好，且兩者之間沒有達顯著性差異。夜間綠燈 LED 照射為物理性的防治方式，雖然沒有使用任何化學農藥，但防治荔枝細蛾的效果與傳統的噴藥方式相當，為相當有效的防治方式。夜間自動噴霧防治荔枝細蛾的為害率 10%，雖然與不噴藥對照組達顯著差異，但農民與消費者對果實為害的期望接近 0%，所以，此套自動噴霧系統對於防治效率的提升仍有改善空間。

自動噴霧系統雖可減少噴藥人力成本，但因荔枝產業對荔枝細蛾防治效率的要求極高，單獨使用尚不易達到產業端對荔枝細蛾為害率的要求。因果實生長期間仍需有使用殺菌藥防治炭疽病或露疫病等病害的需求，若使用自動噴霧系統噴施殺菌劑，同時搭配夜間綠光燈照方式防治荔枝細蛾，有機會達成節省人力成本與經濟生產的目標。自動噴霧系統使用於開花率提升仍有加強空間，未來可持續開發穩定荔枝生產栽培技術或以品種更新方式提升開花率，使荔枝產業可在臺灣永續發展。

誌謝

本研究承蒙行政院農業部經費補助，農業部農業試驗所鳳山熱帶園藝試驗分所熱帶果樹系賴秋炫小姐、胡鐸騰先生、馬秀燕小姐及丁漢彥先生協助本研究進行。在此一併誌謝。

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附錄 . 本試驗使用藥劑種類與稀釋倍數。

Appendix. Types of pesticides and dilution ratios used in the experiment.

Application date	Type of pesticide and dilution ratio	
2022/3/28	acetamiprid 2,000 fold	carbosulfan 1,000 fold
2022/4/4	acetamiprid 2,000 fold	carbosulfan 1,000 fold
2022/4/11	fenitrothion 1,000 fold	carbaryl 1,000 fold
2022/4/18	acetamiprid 2,000 fold	fenitrothion 1,000 fold
2022/4/25	fenitrothion 1,000 fold	carbaryl 1,000 fold
2022/5/2	fenthion 1,000 fold	lambda-cyhalothrin 2,000 fold
2022/5/9	fenitrothion 1,000 fold	acetamiprid 2,000 fold
2022/5/16	fenthion 1,000 fold	lambda-cyhalothrin 2,000 fold
2022/5/23	acetamiprid 2,000 fold	lambda-cyhalothrin 2,000 fold

Evaluation of Using Automatic Micro-Spraying Facilities for 'Yu-Her-Pao' Litchi (*Litchi chinensis*) Flower Induction and Litchi Fruit Borer (*Conopomorpha sinensis*) Control

Chih-Cheng Hsu[1], Hsing-Liang Chen[1], and Hsin-Hsiu Fang[2,*]

Abstract

Hsu, C. C., H. L. Chen, and H. H. Fang. 2024. Evaluation of using automatic micro-spraying facilities for 'Yu-Her-Pao' litchi (*Litchi chinensis*) flower induction and litchi fruit borer (*Conopomorpha sinensis*) control. J. Taiwan Agric. Res. 73(4):259–268.

To improve the flowering rate of litchi (*Litchi chinensis* Sonn.), this study used an automatic spraying system to spray water for cooling at different times in December and investigated the flowering rate in the next March. The results showed that the flowering rates of the daytime spraying treatments were significantly higher than that of the non-spraying control group, while the nighttime spraying treatment did not significantly increase the flowering rate. The flowering rates of the four different spraying treatments were 18.3% for non-spraying, 32.1% for daytime and nighttime spraying, 42.9% for daytime spraying, and 26.7% for nighttime spraying. Daytime spraying was the best time to increase litchi flowering rate. The same spraying system was further used to test the effect of pesticide spraying on litchi fruit borer (*Conopomorpha sinensis* Bradley) control. The results showed that the damage rates of the four control treatments were 61% for the non-pesticide control, 10% for the automatic pesticide spraying, 0% for the manual pesticide spraying, and 2% for the night green light-emitting diode (LED) light illumination. Both daytime manual pesticide spraying and night green LED light control had positive effects. Although the automatic pesticide spraying method can save labor and reduce the damage rate of litchi fruit borer, there is still room for pest control improvement.

Key words: Litchi, Flowering rate, Litchi fruit borer, Temperature, Pesticide.

Received: April 19, 2024; Accepted: July 31, 2024.

[*] Corresponding author: show@tari.gov.tw
[1] Assistant Research Fellows, Department of Tropical Fruit Trees, Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaohsiung, Taiwan, ROC.
[2] Associate Research Fellow, Department of Tropical Fruit Trees, Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaohsiung, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 73(4):269–280 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0005 研究報告

不同定植期及採收次數對紫色葉菜甘藷酚類化合物及抗氧化能力之影響

何佳勳 [1] 楊滿霞 [2] 蕭巧玲 [2] 賴永昌 [3] 林慧玲 [4,*]

何佳勳、楊滿霞、蕭巧玲、賴永昌、林慧玲。2024。不同定植期及採收次數對紫色葉菜甘藷酚類化合物及抗氧化能力之影響。台灣農業研究 73(4):269–280。

摘要

本研究以農業部農業試驗所嘉義農業試驗分所育成紫葉品系的葉菜甘藷 CYY84–67 作為試驗材料，並以 2017–2018 年種植在農業部農業試驗所農場之試材進行機能性成分分析。本研究目的旨在探討不同定植期與採收次數對紫色葉菜甘藷酚類化合物與抗氧化能力之影響。研究結果顯示，紫色葉菜甘藷於 2017–2018 年不同採收期之葉片總酚含量為 87.3–269.0 mg gallic acid equivalent g^{-1} fresh weight (FW)，莖部總酚含量為 16.6–41.5 mg gallic acid equivalent g^{-1} FW，葉片總花青素含量為 2.8–21.2 mg g^{-1} FW，莖部總花青素含量為 0.2–2.1 mg g^{-1} FW，葉片總黃酮含量約在 21.9–77.5 mg quercetin equivalent g^{-1} FW，莖部含量則在 5.8–11.7 mg quercetin equivalent g^{-1} FW。在抗氧化能力部分，葉片 2,2-diphenyl-1-picrylhydrazyl (DPPH) 自由基清除能力為 34.6–83.7%，莖部為 3.5–11.4%，而葉片的半致效應濃度 (concentration for 50% of maximal effect; EC_{50}) 為 22.05 μg mL^{-1}，莖部的 EC_{50} 濃度則為 184.08 μg mL^{-1}。在普魯士藍還原力部分，紫色葉菜甘藷之葉片為 10.4–75.9 mg ascorbic acid equivalent g^{-1} FW，莖部之還原力則為 1.2–6.4 mg ascorbic acid equivalent g^{-1} FW。綜合試驗結果顯示，在 3 月 7 日以第 3 次採收之機能性成分與抗氧化能力較佳，在 6 月 28 日與 12 月 7 日定植期以第 1 次採收較佳，在 9 月 5 日定植期則以第 2 次採收較佳。再者，紫色葉菜甘藷之葉片酚類化合物與抗氧化能力具有極顯著正相關。

關鍵詞：紫色葉菜甘藷、定植期、採收次數、酚類化合物、抗氧化能力。

前言

隨著時代的變遷與進步，國人的飲食習慣已從『吃得飽』與『吃得好』進展至『吃出健康』與『吃出樂活』境界。一般民眾已逐漸了解飲食與健康的關聯性，再加上人口老化與醫療保健成本提高等因素，使得國人在飲食方面開始選擇，具有較高機能性成分的食品與營養保健產品。由於植物體內含有豐富的花青素 (anthocyanins)、類胡蘿蔔素 (carotenoids)、黃酮類 (flavonoids) 或其他多酚類化合物 (polyphenols) 等次級代謝產物，這些存在於植物體的天然化合物稱為植化素 (phytochemicals)，其普遍存在於蔬果、穀物、堅果、豆類及茶等天然食物中 (Liu 2003; Xiao & Bai 2019)。酚類化合物是廣泛存在於植物體的次級代謝產物，其衍生物包括黃酮類、酚酸及花青素等，已知蔬果中的酚類化合物具有抗氧化能力，可有效清除自由基防止氧化傷害，因此可作為食品與保健等膳食抗氧化劑 (Balasundram *et al.*

投稿日期：2024 年 7 月 2 日；接受日期：2024 年 9 月 11 日。
* 通訊作者：hllin@dragon.nchu.edu.tw
1 農業部農業試驗所作物組助理研究員。臺灣 臺中市。
2 農業部農業試驗所作物組副研究員。臺灣 臺中市。
3 農業部農業試驗所嘉義農業試驗分所農藝系研究員兼系主任。臺灣 嘉義市。
4 國立中興大學園藝學系教授。臺灣 臺中市。

2006; Babbar *et al.* 2014)。此外，不同種類的植化素對人體具有不同的營養保健功能，如花青素有助於預防多種與自由基有關的疾病，包括糖尿病、高血壓、動脈硬化及癌症等，而黃酮類則具有抗糖尿病、降血脂、調節血管滲透壓、保護心血管、抗衰老及抗發炎等作用 (Hollman & Katan 1997; Ghosh & Konishi 2007; Panche *et al.* 2016)。

葉菜甘藷 (*Ipomoea batatas* L.)，英名為 leafy sweet potato，別名為地瓜葉與過溝菜，屬於旋花科甘藷屬之 1 年或多年生蔓生植物，具有匍匐地面生長特性且一年四季皆可生產。葉菜甘藷富含多酚物質，尤其以咖啡奎寧酸 (caffeoylquinic acids) 與其衍生物具有很高的抗氧化能力 (Kurata *et al.* 2007; Taira *et al.* 2013; Sun *et al.* 2014)。Chu *et al.* (2000) 分析臺灣數種蔬菜之黃酮類含量，發現紫色葉菜甘藷的總黃酮含量 (426.8 mg kg^{-1}) 最高，其中黃酮醇的楊梅黃酮 (myricetin) 與斛皮素 (quercetin) 及黃酮類的葉黃酮 (luteolin) 等含量皆高於多種常見蔬菜，顯示紫色葉菜甘藷為具有豐富多酚類的蔬菜作物。Zhang *et al.* (2020) 利用 HPLC-ESI-MS 檢測，發現葉菜甘藷之酚類化合物含有 7 種咖啡奎寧酸衍生物與 4 種黃酮類，進一步分析葉片與葉柄的多酚含量與抗氧化能力，顯示葉片之總酚與總黃酮含量高於葉柄約 10 倍，且葉片酚類化合物之咖啡奎寧酸衍生物可能是主要的抗氧化成分。再者，Liu *et al.* (2012) 研究指出，葉菜甘藷「台農 71 號」之頂芽與展開葉第一葉，具有最高的總酚含量與 2,2-diphenyl-1-picrylhydrazyl (DPPH) 自由基清除能力，且春作採收期會顯著影響抗氧化能力表現。Liao *et al.* (2011) 研究顯示，‘Tainung 10’ 與 ‘Tainung 57’ 之抗氧化能力表現優於 ‘Tainung 66’ 與黃葉品系，且對 H_2O_2 誘導的細胞毒性也有保護作用，而總酚與黃酮類可能是主要的抗氧化成分。Li *et al.* (2019) 分析 2 種紫色葉菜甘藷品種之花青素，發現含有 9 種醯化型矢車菊花青素與 9 種醯化型芍藥花青素，其中醯基包含咖啡醯 (caffeoyl)、香豆醯 (p-coumaryl)、阿魏醯 (feruloyl) 及羥基苯甲醯 (p-hydroxy benzoyl)，惟花青素含量會因品種而有所差異，且葉片的花青素成分與含量也會與紫藷塊根不同。另一方面，在不同期作環境下甘藷植體之機能性成分也會有所差異，其中葉片之斛皮素含量以秋作最高，而矢車菊素 (cyanidin)、芍藥素 (peonidin) 及葉黃體素 (lutein) 則以春作較高，在莖部之楊梅素 (myricetin) 含量以夏作較高，而矢車菊素則以春作最高 (Chan 2010)。綜合上述研究顯示，葉菜甘藷之品種、部位及採收期環境條件等均會影響機能性成分含量與抗氧化能力表現。

本研究目的旨在探討不同定植期與採收次數對紫色葉菜甘藷不同部位機能性成分的差異，據以篩檢出機能性成分較高之最適定植與採收期，期能作為高機能性成分蔬菜生產模式之理論與應用基礎，並評估紫色葉菜甘藷作為保健食品原料之可行性。

材料與方法

本研究在農業部農業試驗所農場 (臺中市霧峰區) 進行試驗，以嘉義農業試驗分所育成的心形紫葉品系 CYY84–67 作為參試材料，分別於 2017 年 3 月 7 日、6 月 28 日、9 月 5 日及 12 月 7 日定植，每定植 1 次將連續採收 3 次，共計 12 個採收期 (表 1)。本試驗以 15–20 cm 先端苗進行斜插，每次定植之試驗設計採用逢機完全區集設計 (randomized complete block design; RCBD)，田區規劃 4 區集，每小區種植 40 株，每畦長 5 m、寬 8 m，行距 1.2 m，株距固定為 0.5 m，採雙行植，植體採收標準係以紫色葉菜甘藷覆蓋整個畦面始進行採收作業，取樣方式為離畦面 15–20 cm 處修平。試驗田間栽培管理參考 Ho *et al.* (2024) 報告。

機能性成分測定

本試驗參考 Sarpate *et al.* (2009) 之萃取方法經修飾後進行，將採收後的紫色葉菜甘藷分別秤取葉片與莖部各 20 g 鮮重，經液態氮破碎後以 1：20 比例加入含有 1% 1 N 鹽酸的甲醇 (methanol) 於室溫下浸泡 48 h，再用濾紙 (GA-55, Advantec, Tokyo, Japan) 過濾收集濾液並反覆 2 次，將濾液合併經 30℃真空減壓

表 1. 紫色葉菜甘藷 CYY84–67 於 2017–2018 年期間合計 12 個採收期之定植日期、採收次數、採收日期、定植後日數及葉片與莖部甲醇萃取率。

Table 1. Dates of planting, harvest, batch of harvest, days after planting time, and extraction rate from the leaves and stems of 12 cultivation periods of purple leafy sweet potato CYY84–67 in 2017–2018.

Date of planting	Batch of harvest	Date of harvest	Days after planting time	Extraction rate of leaves (%)[z]	Extraction rate of stems (%)
2017/03/07	1	2017/04/20	44	7.3	6.3
	2	2017/05/17	71	10.2	6.7
	3	2017/06/20	105	10.9	6.2
2017/06/28	1	2017/08/11	44	10.9	6.5
	2	2017/09/07	71	8.9	6.3
	3	2017/09/29	93	8.1	6.5
2017/09/05	1	2017/10/12	37	8.7	5.5
	2	2017/11/09	65	9.4	8.0
	3	2017/12/07	93	9.5	6.1
2017/12/07	1	2018/02/26	81	10.5	5.9
	2	2018/03/30	113	9.2	6.3
	3	2018/04/26	140	8.3	5.4

[z] Extraction rate (%) = (extract weight of 100% MeOH + 1% 1N HCl / 20 g fresh weight) × 100%.

濃縮機 (RE400/BM 200, Yamato Scientific Co., Ltd., Tokyo, Japan) 抽乾後，再以冷凍乾燥機 (EYELA FDU-2200, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) 凍乾 24 h，即得本試驗材料萃取物進行機能性成分與抗氧化能力分析。

總酚含量測定 (total phenolic content)

總酚含量測定參考 Kujala *et al.* (2000) 之萃取方法。將每 1 mg 紫色葉菜甘藷萃取物溶於 1 mL 甲醇 (50% methanol)，取 100 µL 萃取液與 100 µL 福林酚試劑 (1 N Folin-Ciocalteu reagent) 混合在 1.5 mL 離心管。將混合物靜置 10 min，再加入 200 µL 碳酸鈉 (20% Na_2CO_3) 溶液並混合均勻。在室溫下靜置 30 min 後，將混合物置於離心機，以 20,000× *g* (13,000 rpm) 離心 10 min。取 200 µL 上清液至 96 微孔盤中，利用分光光度計 (SPECTROstar® Nano, BMG Labtech, Ortenberg, Germany) 測量 730 nm 波長的吸光值。以沒食子酸 (gallic acid) 為標準品，配置 5、10、20、40、80、100 及 200 µg mL^{-1} 濃度範圍以製作檢量線，並換算紫色葉菜甘藷每克鮮重中，所含沒食子酸的相對量毫克數，以 mg GAE g^{-1} (gallic acid equivalent; GAE，沒食子酸當量) 表示。

總黃酮含量測定 (total flavonoid content)

總黃酮含量測定參考 Arvouet-Grand *et al.* (1994) 之萃取方法。將每 1 mg 紫色葉菜甘藷萃取物溶於 1 mL 甲醇 (75% methanol)，取 250 µL 萃取液與 1.4 mL ddH_2O、50 µL 氯化鋁 (10% $AlCl_3$) 及 50 µL 醋酸鉀 (1 M CH_3COOK) 混合在 2 mL 離心管。將混合物在室溫下避光靜置 30 min，取 200 µL 上清液至 96 微孔盤中，利用前述分光光度計測量 415 nm 波長的吸光值。以槲皮素 (quercetin) 為標準品，配置 5、10、20、40、80 µg mL^{-1} 濃度範圍以製作檢量線，並換算紫色葉菜甘藷每克鮮重中，所含槲皮素的相對量毫克數，以 mg QE g^{-1} (quercetin equivalent; QE，槲皮素當量) 表示。

總花青素含量測定 (total anthocyanin content)

以矢車菊素-3-葡萄糖苷 (cyanidin-3-glucoside) 分子量作為計算標準，利用酸鹼值差異法 (pH differential method) 計算總花青素含量 (Ayu *et al.* 2018)。將每 5 mg 紫色葉菜甘藷萃取物溶於 1 mL 萃取液 (100% 甲醇 + 1% 1N 鹽酸)。取 200 µL 萃取液分別與 800 µL 氯化鉀緩衝液 (0.025 M potassium chloride buffer,

pH 1.0) 及 800 µL 醋酸鈉緩衝液 (0.4 M sodium acetate buffer, pH 4.5) 混合在 2 mL 離心管。將混合物在室溫下避光靜置 15 min。取 1 mL 上清液至比色管中，利用分光光度計測量 520 nm 與 700 nm 波長的吸光值，並將測量值帶入下列公式中：

Total anthocyanin contents (mg g^{-1}) = (A × MW × DF × 1,000) / (ε × l × W)

A = ($A_{520\ nm}$ – $A_{700\ nm}$) pH 1.0 – ($A_{520\ nm}$ – $A_{700\ nm}$) pH 4.5

MW：cyanidin-3-glucoside 之分子量，MW = 449.2 g mol^{-1}

DF：dilution factor (稀釋倍數)

ε：molar extinction coefficient，ε = 26,900 L mol^{-1} cm^{-1}

l：光通過的路徑 (1 cm)

W：萃取物的重量 (g)

抗氧化能力分析

DPPH 自由基清除能力 (DPPH scavenging effect)

本測定參考 Aoshima *et al.* (2004) 之分析方法。取 10 µL 樣品萃取物 (濃度為 30 µg mL^{-1}) 與 200 µL DPPH- 甲醇溶液 (0.1 mM) 及 90 µL Tris-HCl 緩衝液 (50 mM, pH 7.4) 混合在 96 微孔盤，對照組為 100% 甲醇溶液。將混合物在室溫下避光靜置 30 min 後，利用分光光度計測量 517 nm 波長的吸光值，再以公式計算：DPPH 自由基清除能力 (%) = [(對照吸光值 – 樣品吸光值)/對照吸光值] × 100%。以抗壞血酸 (ascorbic acid) 為對照組，並配置不同的萃取物濃度以製作檢量線計算半致效應濃度 (concentration for 50% of maximal effect; EC_{50}) 數值，抗壞血酸配置濃度範圍為 0.625、1.25、2.5 及 5 µg mL^{-1}，葉片濃度範圍為 5、10、20、30 及 40 µg mL^{-1}，莖部濃度範圍為 10、25、50、100、200 及 300 µg mL^{-1}，EC_{50} 數值乃清除 50% DPPH 自由基的有效濃度。

普魯士藍還原力 (reducing power)

本測定參考 Singh & Rajini (2004) 之分析方法。將每 1 mg 紫色葉菜甘藷萃取物溶於 1 mL ddH_2O。取 100 µL 萃取液與 100 µL 磷酸鹽緩衝液 (0.2 M phosphate buffer, pH 6.6) 及 100 µL 赤血鹽 (1% potassium ferricyanide) 混合在 1.5 mL 離心管，以乾浴 50℃反應 20 min 後，再以冰塊冷卻。再加入 100 µL 三氯乙酸 (10% trichloroacetic acid) 至離心管後，將混合物置於離心機，以 820× *g* (3,000 rpm) 離心 10 min。取 100 µL 上清液至 96 微孔盤中，再加入 100 µL ddH_2O 與 20 µL 氯化鐵 (0.1% ferric chloride) 混合均勻後，在室溫下反應 10 min，利用分光光度計測量 700 nm 波長的吸光值。以抗壞血酸 (ascorbic acid) 為對照組，並配置葉片與莖部濃度範圍為 1、5、10、25、50、75 及 100 µg mL^{-1} 以比較不同部位之還原力差異。以抗壞血酸 (ascorbic acid) 為標準品，配置上述濃度範圍以製作檢量線，並換算紫色葉菜甘藷每克鮮重中，所含抗壞血酸還原力的相對量毫克數，以 mg AAE g^{-1} (ascorbic acid equivalent; AAE，抗壞血酸當量) 表示。

統計分析與繪圖

以 Bartlett 分析檢定是否可合併 4 個定植試驗，分析結果為葉之黃酮類為均值同質，可合併 4 個定植期作比較；其餘觀測值分析結果為均方非同質，則各定植期分別進行 ANOVA (analysis of variance) 分析。試驗數據皆以均值 ± 標準誤差表示，統計分析之 ANOVA、最小顯著性差異 (Fisher’s protected least significant difference test; LSD test) 及相關矩陣分析係利用 SAS Enterprise Guid 統計軟體 (version 7.1)，而統計繪圖則使用 SPSS 公司之 SigmaPlot 軟體 (version 12.5)。

結果與討論

本研究以嘉義農業試驗分所育成紫葉品系的葉菜甘藷 CYY84–67 作為試驗材料，並以 2017–2018 年種植在農業部農業試驗所農

場之試材進行機能性成分分析。本試驗分別於 2017 年 3 月 7 日、6 月 28 日、9 月 5 日及 12 月 7 日定植，每定植 1 次將連續採收 3 次，共計 12 個採收期 (表 1)。將不同採收期的紫色葉菜甘藷之葉片與莖部分別秤取 20 g 鮮重，經液態氮破碎後以 1：20 比例加入含有 1% 1 N 鹽酸的甲醇進行萃取，通過真空減壓濃縮機抽乾後，再以真空冷凍乾燥機凍乾，即得試驗材料萃取物。由表 1 結果顯示，紫色葉菜甘藷 CYY84–67 於 2017–2018 年不同採收期之葉片甲醇萃取率約 7.3–10.9%，而莖部為 5.4–8.0%，其中以葉片萃取率高於莖部。為探討不同定植期與採收次數對紫色葉菜甘藷不同部位機能性成分之差別效應，分別將上述試驗材料萃取物分析總酚 (total phenolic content; TPC)、總花青素 (total anthocyanin content; TAC) 及總黃酮 (total flavonoid content; TFC) 含量變化。

由表 2 結果顯示，紫色葉菜甘藷於 2017–2018 年不同採收期之葉片 TPC 為 87.3–269.0 mg GAE g^{-1} fresh weight (FW)，莖部為 16.6–41.5 mg GAE g^{-1} FW；葉片 TAC 為 2.8–21.2 mg g^{-1} FW，莖部為 0.2–2.1 mg g^{-1} FW；葉片 TFC 約在 21.9–77.5 mg QE g^{-1} FW，莖部約在 5.8–11.7 mg QE g^{-1} FW。在抗氧化能力結果顯示，葉片 DPPH 自由基清除能力為 34.6–83.7%，莖部為 3.5–11.4%，而葉片還原力為 10.4–75.9 mg AAE g^{-1} FW，莖部為 1.2–6.4 mg AAE g^{-1} FW (表 3)，初步比較上述機能性成分含量及抗氧化能力部分，葉片皆明顯高於莖部。

以 Bartlett 分析檢定是否可合併 4 個定植試驗比較不同採收次數之機能性成分及抗氧化能力，分析結果為葉之黃酮類 (TFC) 為均方同質，可合併 4 個定植期作比較；其餘觀測值分析結果為均方非同質，則各定植期分別進行資料分析。由試驗結果發現，葉片之 TPC、TAC 及 TFC 表現，在 3 月 7 日定植期以第 1 次採收期最低，第 3 次採收期最高，且兩者達顯著差異；在 12 月 7 日定植期，以第 1 次採收期最高，且與第 2 及 3 次採收期有顯著差異；6 月 28 日定植期以第 1 次採收期平均值最高，其中 TPC 及 TFC 達顯著差異；9 月 5 日定植期以第 2 次採收期最高，其中 TAC 達顯著差異 (表 2)。葉片之黃酮類 (TFC) 可合併 4 個定植期比較結果，以 6 月 28 日定植期之第 1 次採收期含量最高，而 3 月 7 日定植期之第 1 次採收含量最低 (表 4)。在抗氧化能力部分，葉片之 DPPH 自由基清除能力與前面 4 項觀測值趨勢不完全相同，相同部分為 12 月 7 日定植期仍以第 1 次採收期最高，且與第 2 及 3 次採收期有顯著差異，6 月 28 日定植期則以第 1 次採收期最高，9 月 5 日定植期皆以第 2 次採收期最高；相異部分為在 3 月 7 日定植期以第 2 次採收期最高 (表 3)。此外，不同定植期之還原力表現與機能性成分具有相同趨勢 (表 3)。綜合上述結果，在 3 月 7 日以第 3 次採收之機能性成分與抗氧化能力較佳，在 6 月 28 日與 12 月 7 日定植期以第 1 次採收較佳，9 月 5 日定植期則以第 2 次採收較佳。另一方面，莖部以第 1 與 2 次採收之機能性成分或抗氧化能力有較高的趨勢 (表 2 與表 3)。

已知溫度、光照及季節性環境均會影響植體酚類化合物生合成表現，其中光輻射和溫度與酚酸及黃酮類含量具有正相關，而花青素與總酚的累積與季節溫度呈負向變化 (Borochv-Neori *et al.* 2011; Marin *et al.* 2015; Alba *et al.* 2022)。Chan (2010) 論文顯示，甘藷葉片之斛皮素含量以秋作最高，而矢車菊素、芍藥素與葉黃體素則以春作較高，因此在不同期作環境下甘藷植體之機能性成分會有所差異。Ghorbanli *et al.* (2012) 研究發現，附生地衣 *Flavoparmelia caperata* 之酚類化合物、黃酮及花青素在冬季環境下會顯著地增加，而 *Physcia dubia* 在夏季會促進酚類化合物表現，冬季則會使花青素含量增加。Zhang *et al.* (2019) 研究指出，小花蔓澤蘭 (*Mikania micrantha*) 在冬季低溫環境下，會促進葉與莖中花青素的累積，此現象乃是提高其抗氧化能力以增加冬季低溫的耐受性。本試驗結果發現，葉片 TFC 為合併 4 個定植期分析結果，定植期與採收次數交感顯著，故比較定植期與採收次數兩因子之所有處理組合，以 6 月 28

表 **2.** 比較紫色葉菜甘藷 CYY84–67 葉片與莖部在 4 個定植期連續採收 3 次之酚類化合物。

Table 2. Comparisons of phenolic compounds from the leaves and stems of purple leafy sweet potato CYY84–67 among 3 consecutive harvests in 4 planting dates.

	Leaves				Stems			
Batch of harvest	2017/03/07	2017/06/28	2017/09/05	2017/12/07	2017/03/07	2017/06/28	2017/09/05	2017/12/07
TPC[z] (mg GAE g^{-1} FW)								
1	107.2 ± 13.4 b[y]	261.2 ± 10.3 a	176.8 ± 15.6 a	269.0 ± 13.0 a	31.3 ± 0.7 a	41.5 ± 1.3 a	25.0 ± 4.1 b	35.4 ± 2.7 a
2	202.8 ± 17.4 a	170.8 ± 11.4 b	213.5 ± 28.5 a	98.8 ± 9.4 b	30.0 ± 0.8 a	36.4 ± 3.7 ab	41.0 ± 6.1 a	22.4 ± 0.3 b
3	244.3 ± 14.2 a	158.9 ± 8.3 b	166.3 ± 11.1 a	87.3 ± 6.0 b	30.0 ± 3.2 a	29.2 ± 2.6 b	19.2 ± 0.7 b	16.6 ± 1.0 b
TAC (mg g^{-1} FW)								
1	3.6 ± 0.7 c	16.2 ± 1.5 a	12.4 ± 1.5 b	21.2 ± 1.9 a	1.2 ± 0.0 b	1.5 ± 0.1 a	0.8 ± 0.1 b	2.1 ± 0.1 a
2	8.7 ± 0.9 b	12.1 ± 1.1 a	19.8 ± 2.9 a	5.2 ± 0.9 b	0.5 ± 0.0 c	1.1 ± 0.0 b	2.1 ± 0.1 a	0.4 ± 0.1 b
3	17.8 ± 1.0 a	13.9 ± 0.6 a	19.1 ± 2.8 ab	2.8 ± 0.6 b	2.1 ± 0.1 a	1.2 ± 0.2 b	0.3 ± 0.0 c	0.2 ± 0.0 b
TFC (mg QE g^{-1} FW)								
1	21.9 ± 3.0 b	77.5 ± 5.8 a	50.8 ± 5.2 a	60.5 ± 4.3 a	6.1 ± 0.6 b	7.5 ± 0.4 a	7.4 ± 0.7 b	7.4 ± 0.7 a
2	52.2 ± 4.1 a	56.5 ± 2.8 b	63.3 ± 7.5 a	43.0 ± 4.3 b	9.2 ± 0.7 a	8.0 ± 0.8 a	11.7 ± 1.3 a	6.4 ± 0.4 a
3	52.2 ± 1.8 a	50.6 ± 2.7 b	46.8 ± 3.6 a	43.1 ± 4.4 b	7.9 ± 0.7 ab	7.3 ± 0.4 a	6.8 ± 1.0 b	5.8 ± 0.4 a

[z] TPC: total phenolic content; GAE: gallic acid equivalent; FW: fresh weight; TAC: total anthocyanin content; TFC: total flavonoid content; QE: quercetin equivalent.

[y] Mean ± standard error (n = 8). Means within a column followed by the same letter(s) are not significantly different at 5% level by Fisher's protected least significant difference (LSD) test.

表 3. 比較紫色葉菜甘藷 CYY84–67 葉片與莖部在 4 個定植期連續採收 3 次之抗氧化能力。

Table 3. Comparisons of antioxidant capacity from the leaves and stems of purple leafy sweet potato CYY84–67 among 3 consecutive harvests in 4 planting dates.

Batch of harvest	Leaves				Stems			
	2017/3/7	2017/6/28	2017/9/5	2017/12/7	2017/3/7	2017/6/28	2017/9/5	2017/12/7
DPPH scavenging effect (%)[z]								
1	55.5 ± 2.0 c[y]	83.1 ± 0.8 a	67.7 ± 1.4 b	82.4 ± 1.6 a	11.4 ± 0.1 a	9.4 ± 0.2 b	8.6 ± 0.4 a	10.0 ± 0.9 a
2	72.0 ± 2.5 a	68.3 ± 1.8 b	83.7 ± 0.8 a	46.1 ± 2.2 b	8.9 ± 0.2 c	10.6 ± 0.3 a	9.0 ± 0.6 a	6.0 ± 0.2 b
3	61.1 ± 1.6 b	67.6 ± 1.6 b	61.2 ± 2.6 b	34.6 ± 1.5 c	9.5 ± 0.2 b	6.9 ± 0.5 c	5.9 ± 0.2 b	3.5 ± 0.5 c
Reducing power (mg AAE g^{-1} FW)								
1	22.8 ± 2.8 b	65.6 ± 4.0 a	40.9 ± 4.8 a	75.9 ± 6.6 a	6.1 ± 0.4 a	6.4 ± 0.6 a	2.8 ± 0.3 b	5.2 ± 0.2 a
2	47.0 ± 5.9 a	38.5 ± 2.0 b	60.6 ± 7.9 a	23.3 ± 1.6 b	3.7 ± 0.1 b	5.0 ± 0.5 ab	5.7 ± 0.7 a	1.9 ± 0.2 b
3	51.8 ± 1.3 a	39.4 ± 3.6 b	43.1 ± 3.5 a	10.4 ± 0.7 b	5.7 ± 0.6 a	3.9 ± 0.5 b	1.5 ± 0.2 c	1.2 ± 0.1 c

[z] DPPH: 2,2-diphenyl-1-picrylhydrazyl; AAE: ascorbic acid equivalent; FW: fresh weight.
[y] Mean ± standard error (n = 8). Means within a column followed by the same letter(s) are not significantly different at 5% level by Fisher's protected least significant difference (LSD) test.

表 4. 紫色葉菜甘藷 CYY84–67 葉片之總黃酮綜合變方分析交感效應。

Table 4. Sympathetic effects of the total flavonoid content comprehensive variance analysis in the leaves of purple leafy sweet potato CYY84–67.

Date of planting	Batch of harvest	Mean ± SE
2017/6/28	1	77.5 ± 5.8 a
2017/9/5	2	63.3 ± 7.5 b
2017/12/7	1	60.5 ± 4.3 b
2017/6/28	2	56.5 ± 2.8 bc
2017/3/7	3	52.2 ± 1.8 bcd
2017/3/7	2	52.2 ± 4.1 bcd
2017/9/5	1	50.8 ± 5.2 bcd
2017/6/28	3	50.6 ± 2.7 bcd
2017/9/5	3	46.8 ± 3.6 cd
2017/12/7	3	43.1 ± 4.4 d
2017/12/7	2	43.0 ± 4.3 d
2017/3/7	1	21.9 ± 3.0 e

[x] Mean ± standard error (n = 8). Different letters mean significant differences at 5% level by Fisher's protected least significant difference (LSD) test.

日定植期之第 1 次採收期含量最高，而 3 月 7 日定植期之第 1 次採收含量最低 (表 4)。紫色葉菜甘藷在 2017 年 12 月 7 日定植期之第 1 次採收，即 2018 年 2 月 26 日採收期之 TPC、TAC 及 TFC 表現最佳 (表 2)，顯示低溫可能會誘導 TPC、TAC 及 TFC 增加。先前試驗結果顯示，2017 年 12 月 7 日定植至第 1 次採收之平均氣溫為 17℃，且在此生育期間氣溫低於 15℃的天數約有 21 d (Ho *et al.* 2024)，推測低溫可能會導致紫色葉菜甘藷之酚類化合物含量累積。

本研究進一步以紫色葉菜甘藷 CYY84–67 在 2018 年 2 月 26 日採收期，分析葉片與莖部不同濃度萃取物之 DPPH 自由基清除能力檢量線及 EC_{50} 濃度，對照組為抗壞血酸。由圖 1 結果顯示，紫色葉菜甘藷 CYY84–67 之葉片與莖部不同濃度萃取物與 DPPH 自由基清除能力呈顯著線性關係，利用線性方程式計算抗壞血酸的 EC_{50} 濃度為 2.95 μg mL^{-1}、葉片的 EC_{50} 濃度為 22.05 μg mL^{-1}，而莖部的 EC_{50} 濃度為 184.08 μg mL^{-1}，結果可知抗壞血酸之 DPPH 自由基清除能力最強，其次是葉片而莖

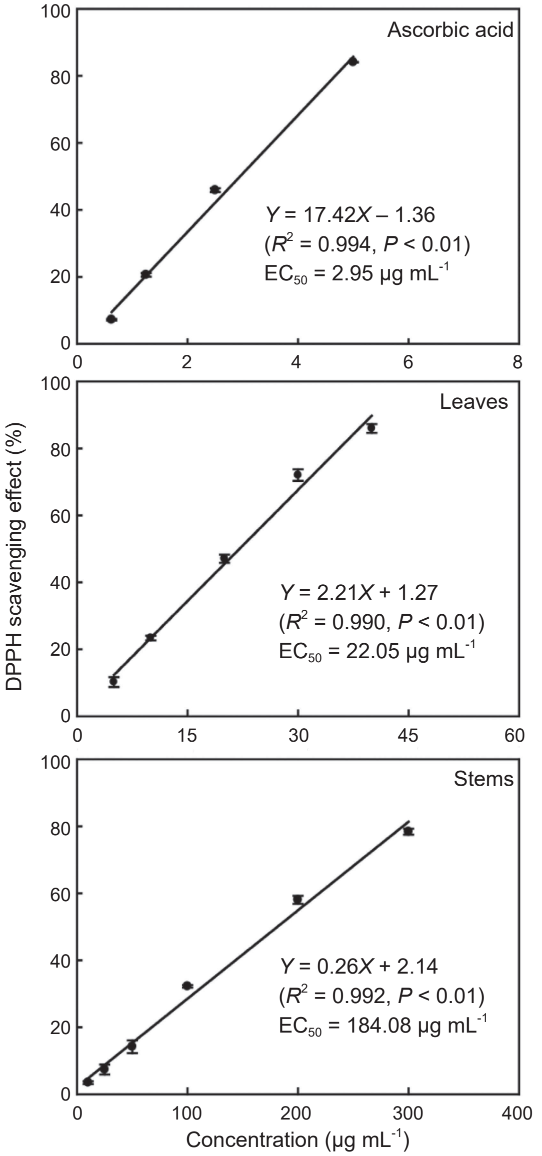

圖 1. 紫色葉菜甘藷 CYY84–67 於 2018 年 2 月 26 日採收日期下葉片與莖部不同濃度萃取物之 2,2-diphenyl-1-picrylhydrazyl (DPPH) 自由基清除能力檢量線及 EC_{50} 濃度，對照組為抗壞血酸。

Fig. 1. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect calibration curve and EC_{50} from leaves and stems with different concentrations of purple leafy sweet potato CYY84–67 in February 26, 2018, and control is ascorbic acid.

部表現最差。已知普魯士藍還原力旨在將赤血鹽 ($K_3Fe(CN)_6$) 還原成黃血鹽 ($K_4Fe(CN)_6$)，而黃血鹽再與 Fe^{3+} 作用以生成普魯士藍，通過檢測普魯士藍生成量，以評估參試樣品作為抗氧化物的還原力 (Yıldırım *et al.* 2001)。本研究同樣取 2018 年 2 月 26 日採收期，分析葉片與莖部之不同濃度萃取物在 700 nm 波長的吸光讀值，對照組為抗壞血酸。由圖 2 結果發現，抗壞血酸的還原力最高，其次為紫色葉菜甘藷之葉片及莖部萃取物。進一步將紫色葉菜甘藷 CYY84–67 之機能性成分與抗氧化能力進行相關矩陣分析，結果顯示葉片的機能性成分與抗氧化能力具有極顯著正相關，其中又以總酚與總花青素及抗氧化能力的係數較高，而莖部的總酚與總花青素及抗氧化能力亦有極顯著正相關 (表 5)。

已知葉菜甘藷含有多酚物質，尤其是咖啡奎寧酸及其衍生物具有很高的抗氧化能力 (Kurata *et al.* 2007; Taira *et al.* 2013; Sun *et al.* 2014; Zhang *et al.* 2020)，而紫色葉菜甘藷

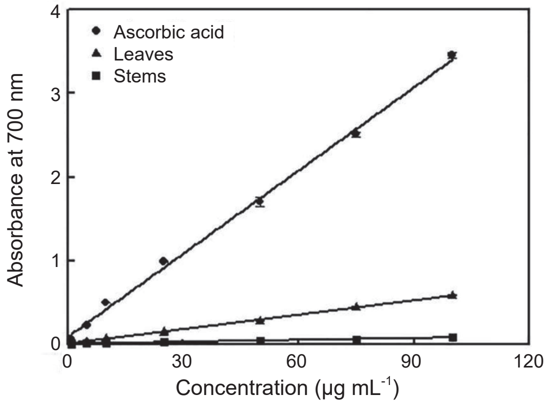

圖 2. 紫色葉菜甘藷 CYY84–67 於 2018 年 2 月 26 日採收日期，葉片與莖部不同濃度萃取物之 700 nm 波長的吸光值，對照組為抗壞血酸。

Fig. 2. The absorbance reading at 700 nm from leaves and stems with different concentrations of purple leafy sweet potato CYY84–67 in February 26, 2018, and control is ascorbic acid.

表 5. 紫色葉菜甘藷 CYY84–67 葉片與莖部萃取物的酚類化合物與抗氧化能力之相關矩陣係數。

Table 5. Correlation matrix with the Pearson coefficient values for phenolic compounds and antioxidant ability of the leaves and stems extracts in purple leafy sweet potato CYY84–67.

Parameter	TPC[z]	TFC	TAC	AOA (DPPH)	AOA (Reducing power)
Leaves of CYY84–67 (n = 48)					
TPC	1	0.491**	0.755**	0.864**	0.906**
TFC		1	0.549**	0.558**	0.530**
TAC			1	0.701**	0.808**
AOA (DPPH)				1	0.919**
AOA (Reducing power)					1
Stems of CYY84–67 (n = 48)					
TPC	1	0.226	0.675**	0.724**	0.805**
TFC		1	0.299*	0.295*	0.191
TAC			1	0.637**	0.795**
AOA (DPPH)				1	0.825**
AOA (Reducing power)					1

[z] TPC: total phenolic content; TFC: total flavonoid content; TAC: total anthocyanin content; AOA: antioxidant activity according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assays.
** Correlation is significant at $P \leq 0.01$; * correlation is significant at $P \leq 0.05$.

則含有酚類化合物、黃酮類及花青素等成分，具有抗氧化功能可有效抑制發炎作用與預防癌症等潛力 (Chu *et al.* 2000; Lee *et al.* 2015; Li *et al.* 2019)。本研究發現紫色葉菜甘藷之葉片 DPPH 自由基清除能力 EC_{50} 為 22.05 μg mL^{-1}，而莖部的 EC_{50} 濃度為 184.08 μg mL^{-1} (圖 1)，顯示葉片的抗氧化能力較佳。文獻研究指出，植體內機能性成分的組成及含量與抗氧化能力間具有顯著正相關，如小苜蓿 (*Medicago minima*) 葉和根的抗氧化能力與酚類及黃酮類含量有顯著正相關 (Ge & Ma 2013; Kabtni *et al.* 2020; Lee *et al.* 2020)。此外，Liu *et al.* (2012) 研究發現，葉菜甘藷「台農 71 號」之葉片總酚含量愈高，其 DPPH 自由基清除能力愈佳。本研究相關矩陣分析顯示，紫色葉菜甘藷 CYY84–67 葉片的機能性成分與抗氧化能力呈極顯著正相關，其中又以總酚與總花青素及抗氧化能力的係數較高，即總酚與總花青素含量愈多則抗氧化能力愈強 (表 5)。

綜合上述研究顯示，紫色葉菜甘藷含有總酚及花青素，可視為物美價廉的機能性蔬菜，由於紫色葉菜甘藷生長快速且栽培容易，再加上耐旱耐濕與抗病蟲害等特性，因此農藥施用較少亦屬於健康蔬菜，尤其適合機械收穫可大面積栽培。惟 CYY84–67 紫葉品系由於適口性不佳，故作為蔬菜供消費者食用的接受度不高，但其所含的機能性成分，則可提供作為本土保健食品原料之新選擇，以期能開發符合市場需求並具保健功能之新產品，藉此提升紫色葉菜甘藷之多元利用性與附加價值，並增進農民收益以及提供消費者之營養保健參考。

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Effects of Planting Periods and Number of Harvesting Times on Phenolic Compounds and Antioxidant Capacity of Purple Leafy Sweet Potato

Chia-Hsun Ho[1], Man-Hsia Yang[2], Chiao-Ling Hsiao[2], Yung-Chang Lai[3], and Huey-Ling Lin[4,*]

Abstract

Ho, C. H., M. H. Yang, C. L. Hsiao, Y. C. Lai, and H. L. Lin. 2024. Effects of planting periods and number of harvesting times on phenolic compounds and antioxidant capacity of purple leafy sweet potato. J. Taiwan Agric. Res. 73(4):269–280.

In this study, the experimental material of purple leafy sweet potato strain CYY84–67 was bred by Chiayi Agricultural Experiment Branch of Taiwan Agricultural Research Institute (TARI), and the field experiment and functional ingredients analyses were conducted at TARI in 2017 and 2018. The objectives of this study were to investigate the effects of planting periods and number of harvesting times on phenolic compounds and antioxidant capacity of purple leafy sweet potato. The results showed that functional ingredients were present in the leaves and stems of purple leafy sweet potato CYY84–67 at different harvest periods during 2017–2018. The total phenolic content in leaves ranged from 87.3–269.0 mg gallic acid equivalent g^{-1} fresh weight (FW), while the content in stems ranged from 16.6–41.5 mg gallic acid equivalent g^{-1} FW. Leaves had a total anthocyanin content of 2.8–21.2 mg g^{-1} FW, while stems had a content of 0.2–2.1 mg g^{-1} FW. The total flavonoid content in leaves was between 21.9 and 77.5 mg quercetin equivalent g^{-1} FW, while the content in stems was between 5.8 and 11.7 mg quercetin equivalent g^{-1} FW. Regarding antioxidant capacity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect of the leaves was 34.6–83.7%, while that of the stems was 3.5–11.4%. The concentration for 50% of maximal effect (EC_{50}) of the leaves was 22.05 μg mL^{-1}, and the EC_{50} concentration of the stems was 184.08 μg mL^{-1}. The reducing power from the leaves of purple leafy sweet potato CYY84–67 was 10.4–75.9 mg ascorbic acid equivalent g^{-1} FW, while the stems had 1.2–6.4 mg ascorbic acid equivalent g^{-1} FW. The results showed that the functional ingredients and antioxidant capacity were better in the third harvest on March 7. For the planting periods on June 28 and December 7, the first harvest was the best, while for the planting period on September 5, the second harvest was the superior. Furthermore, there was a significant positive correlation between the antioxidant capacity and the phenolic compounds in the leaves of purple leafy sweet potato.

Key words: Purple leafy sweet potato, Planting period, Number of harvesting times, Phenolic compound, Antioxidant capacity.

Received: July 2, 2024; Accepted: September 11, 2024.

[*] Corresponding author, e-mail: hllin@dragon.nchu.edu.tw
[1] Assistant Research Fellow, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[2] Associate Research Fellows, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[3] Research Fellow and Division Director, Department of Agronomy, Chia-Yi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[4] Professor, Department of Horticulture, National Chung Hsing University, Taichung City, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 73(4):281–291 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0006

研究報告

抽穗後高溫處理對水稻花粉活力及稔實率之影響

夏奇鋕 [1] 李長沛 [2] 蔡嫺婷 [3] 蔡挹恆 [4] 黃佩瑩 [4] 莊淨 [5] 游舜期 [5,*]

摘要

夏奇鋕、李長沛、蔡嫺婷、蔡挹恆、黃佩瑩、莊淨、游舜期。2024。抽穗後高溫處理對水稻花粉活力及稔實率之影響。台灣農業研究 73(4):281–291。

本研究在水稻抽穗至穀粒成熟期間於檢定圃進行高溫處理，探討高溫對花粉活力與稔實率之影響。水稻材料為耐熱早熟秈型陸稻 ‘Nagina 22’ (‘N22’)、中晚熟粳稻「台南 11 號」(‘Tainan 11’; ‘TN11’) 與「台粳 9 號」(‘Taiken 9’; ‘TK9’) 以及以 ‘TK9’ 引入 ‘N22’ 耐熱基因育成之近同源系 (BC_4F_5) PA163 與 PA164，於 2020 年第二期作與 2021 年第一期作以水田栽培方式分別種植於田區與耐熱性檢定圃 (塑膠棚溫室)。2020 年第二期作檢定圃於 ‘N22’ 抽穗後至穀粒成熟期間每日 08:00–15:00 以柴油加熱器加熱，設定溫度 38℃；2021 年第一期作白天加熱溫度增為 39℃，並於夜間 20:00 至隔日 06:00 設定加熱溫度為 28℃。植株於抽穗日掛牌並於 2 d 後採穗，取採穗當日即將開放穎花之花粉以電阻抗式流式細胞儀 (impedance flow cytometry; IFC) 檢測花粉活力。2020 年第二期作結果顯示，同一品種 (系) 其田區與檢定圃之花粉活力相較，差異皆不顯著；稔實率相較，亦皆差異不顯著。2021 年第一期作結果顯示，PA164、‘TN11’ 及 ‘TK9’ 之田區與檢定圃花粉活力相較差異顯著；稔實率方面，除上述 3 品種 (系) 外，另有 PA163 亦顯示差異顯著。此外，2021 年第一期作自田區取 5 品種 (系) 穎花之花粉以 25–45℃溫度處理並檢測花粉活力變化，將不同溫度處理測得之花粉活力與其 25℃處理之花粉活力 (最高值) 相較之百分比作為相對花粉活力，並依品種 (系) 相對花粉活力受溫度影響程度由小至大排序，分別為 ‘N22’、‘TN11’、PA164、PA163 及 ‘TK9’。本研究結果顯示花粉活力與稔實率皆受到高溫影響而下降，但採用檢定圃高溫處理成本較高且溫度設定較不具彈性，而採用溫度處理田區花粉並檢測其活力變化，具成本低且實施簡易具彈性之優點，應可作為耐熱水稻種原快速或初級篩選之用。

關鍵詞：水稻、稔實率、花粉活力、電阻抗式流式細胞儀。

前言

水稻為熱帶與亞熱帶作物，是東亞地區最重要的主要糧食，隨著全球氣候暖化趨勢加劇，水稻受到高溫危害的情形益趨頻繁與嚴重，耐熱水稻品種的育成有其必要與重要性 (Chuang & Lur 2013)。臺灣耐熱水稻的篩選常利用一期作田間之自然高溫配合分期播種或延遲種植，達到從生長至收穫皆暴露於高溫環境之目的，但此法受限試驗場域之自然條件對環境因子的強度無法完全掌控。因此，利用設施精準管控環境因子的策略因應而生，其中利用人工氣候室與生長箱以盆栽栽培水稻，雖然環境因子得以控制，但常受限於場域無法採行水田栽培，且盆栽數量亦受到限制 (Hsuan *et al*. 2019)。農業部農業試驗所 (農試所) 利用改良式雙拼圓頂力霸塑膠型溫室加裝柴油加熱設備，在設施內以水田方式栽培水稻，除溫度

投稿日期：2024 年 7 月 2 日；接受日期：2024 年 9 月 23 日。
* 通訊作者：yousc_1@tari.gov.tw
[1] 農業部農業試驗所遺傳資源及生物技術組研究員。臺灣 臺中市。
[2] 農業部農業試驗所作物組研究員。臺灣 臺中市。
[3] 農業部農業試驗所遺傳資源及生物技術組聘用副研究員。臺灣 臺中市。
[4] 農業部農業試驗所遺傳資源及生物技術組計畫助理。臺灣 臺中市。
[5] 農業部農業試驗所遺傳資源及生物技術組助理研究員。臺灣 臺中市。

可調控外，其他栽培條件仍保持與當地田間環境最大之相似度 (光強度與日照時間等)，是現階段觀察高溫逆境對水稻生長、生理、生殖、產量及穀粒品質影響的較佳選擇。

Matsui *et al*. (2001a) 研究指出高溫導致稔實率降低的主因，一為柱頭之花粉數量 (pollen reception) 偏低，二為花粉在柱頭上萌發不良所致，亦即高溫對生殖過程的影響可藉由觀察花粉特性受到高溫影響的改變來評估。傳統上對花粉活力的評估方法有觀察柱頭上之花粉數或花粉管伸長情形等，這些方法大都需要藉由固定組織、染色或切片等操作 (Shi *et al*. 2017)，此外亦可利用花粉發芽培養來評估花粉活力 (Khatun & Flowers 1995; Song *et al*. 2001)。但是，上述諸法皆需較多的執行人力，且觀察數量受限，因此較適合作為現象解釋而非大量篩選之用。本研究過去利用電阻抗式流式細胞儀 (impedance flow cytometry; IFC) 建立水稻花粉活力檢測方法，結果顯示與慣用之花粉培養法或 FDA 染色檢測法並無差異。惟 IFC 前置作業簡單且可檢測之花粉數量遠高於上述方法，在節省人力、時間與提高效率方面皆具優勢 (Hsia *et al*. 2022)。

本研究利用農試所建立的水稻耐熱性檢定圃以水田方式栽培水稻，並於抽穗開始至穀粒成熟期間進行高溫處理，探討不同品種 (系) 水稻之花粉活力與稔實率在高溫影響下的表現。同時以取自田區之水稻花粉進行不同溫度處理，並以 IFC 檢測花粉活力受溫度影響之變化。

材料與方法

水稻材料

供試之 5 個水稻品種 (系) 分別為耐熱早熟之秈型陸稻 (aus type) ‘Nagina 22’ (‘N22’)、中晚熟粳稻「台南 11 號」(‘Tainan 11’; ‘TN11’) 與「台粳 9 號」(‘Taiken 9’; ‘TK9’) 以及利用 ‘TK9’ 導入 ‘N22’ 耐熱基因所育成之近同源系 (BC_4F_5) PA163 與 PA164。

高溫處理

水稻種植地點位於臺中市霧峰區農試所 (經緯度為 120.6881°E, 24.0313°N 海拔高度為 90 m)，以水田栽培方式分別種植於田區及耐熱性檢定圃溫室內，溫室結構為改良式雙拼圓頂力霸塑膠型溫室。田區與檢定圃均採逢機完全區集設計 (randomized complete block design; RCBD)，每品種 4 重複，每重複種植 45 株。2020 年第二期作在早熟稻 ‘N22’ 抽穗後至穀粒成熟期間進行高溫處理，於每日 08:00 開啟柴油加熱器持續加熱至 15:00，設定加熱溫度為 38℃；2021 年第一期作白天設定高溫為 39℃外，於 20:00 至隔日 06:00 設定夜溫為 28℃。試驗期間記錄兩期作各品種之採穗日期 (表 1)、抽穗至穀粒成熟期間 (加熱期間) 田區與檢定圃內的日均溫度變化 (圖 1)，以及田區與檢定圃在加熱時段與未加熱時段之平均溫度 (表 2)。

花粉取樣與活力檢測

選取剛突出劍葉葉枕之稻穗加以標記 (第

表 1.　5 個水稻品種 (系) 於高溫處理溫室 2 連續期作之採穗日期、採穗時溫度以及採穗當日最高溫度。

Table 1.　Panicle harvesting dates, temperatures at picking time, and the highest temperatures on that day for 5 rice varieties (lines) grown in 2 consecutive cropping seasons in a heated greenhouse.

	Second cropping season, 2020			First cropping season, 2021		
Cultivar	Panicle harvesting dates	Temperature at harvesting (℃)	Highest temperature in harvesting day (℃)	Panicle harvesting dates	Temperature at harvesting (℃)	Highest temperature in harvesting day (℃)
‘N22’	10/14	39.7	42.5	4/28	39.2	38.6
PA164	10/16	38.8	42.6	5/19	42.1	44.4
‘Tainan 11’	10/20	39.2	42.0	5/24	42.1	41.1
‘Taiken 9’	10/21	39.4	40.2	5/21	42.6	45.6
PA163	10/22	38.1	39.6	5/20	42.4	44.0

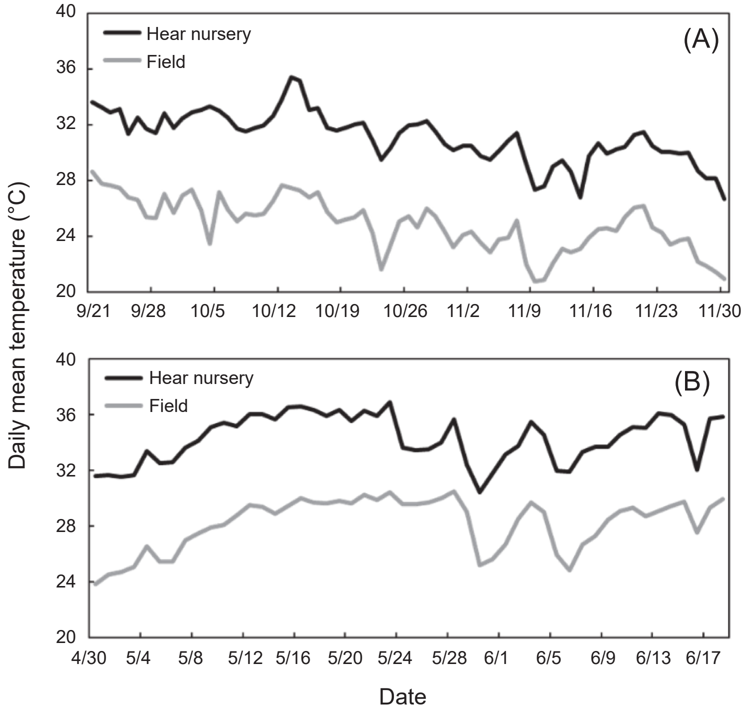

圖 1. (A) 2020 年第二期作與 (B) 2021 年第一期作於田間及高溫處理溫室之平均溫度曲線。

Fig. 1. Mean daily temperature curves of the field and the heating greenhouse in (A) the second cropping season of 2020 and (B) the first cropping season of 2021.

表 2. 田間與高溫處理溫室於 2 連續期作之日均溫以及加溫與未加溫時段的平均溫度。

Table 2. The mean temperatures of the field and the heating greenhouse in the heated and unheated periods in 2 consecutive cropping seasons of this study.

Year-cropping season	Treatment	Mean temperature (℃) 08/03–09/20, 2020			Mean temperature (℃) 09/21–11/30, 2020		
		00:00–24:00	08:00–15:00	15:00–08:00	00:00–24:00	08:00–15:00	15:00–08:00
2020-2	Field	28.2	31.5	27.0	24.8	28.3	23.4
	Heating greenhouse	32.8	36.0	31.5	31.1	37.0	28.6
	Difference	4.6	4.5	4.5	6.3	8.7	5.2
2021-1		03/07–04/20, 2021			04/21–06/20, 2021		
	Field	22.3	25.6	19.9	27.5	30.4	25.6
	Heating greenhouse	27.0	31.1	24.2	33.8	40.4	29.6
	Difference	4.7	5.5	4.2	6.3	10.0	4.0

0 日)，每一品種 (系) 共標記 10 株 (1 穗/株)，於第 2 日選取其中 5 株進行採穗。2020 年第二期作採穗時間為 09:00；2021 年第一期作為 10:00。將採穗株由稻稈基部剪下並置入水杯中再次修剪，將置有稻穗之水杯攜回置於窗邊或光照下促進開穎。取主穗枝前段 (約 6 cm) 將開裂之穎花 5 朵，以鑷子取出花藥放入裝有 500 μL AF6 緩衝液 (Amphasys, Root, Switzerland) 之微量離心管中，以組織研磨棒輕壓花藥釋出花粉後，以 100 μm 濾網過濾，取 200 μL 花粉濾液加入 AF6 緩衝液定量至 500 μL，作為單次分析樣品 (1 重複)，共進行 4 重

複。花粉活力以 IFC Ampha Z30 (Amphasys, Root, Switzerland) 進行檢測，使用 120 µm 孔徑晶片，電波頻率為 12 MHz、馬達轉速 35 rpm，其他檢測參數 modulation/amplification/demodulation 為 4/6/2，trigger level 為 0.1。分析顯示之 X 軸相位角設定為 180–270，Y 軸震幅設定為 0–6，並以 AmphaSoft 2.0 (Amphasys, Root, Switzerland) 軟體進行分析，花粉活力為計算具活力之花粉數占總檢測花粉數之百分比 (Hsia *et al*. 2022)。

田區花粉以不同溫度處理對花粉活力之影響

2021 年第一期作於 10:00 進行上所述 5 個品種 (系) 田區稻穗取樣，每一品種 (系) 選取 6 株，每株取 1 穗，每穗取 6 朵穎花，混合 2 穗共 12 朵穎花之花藥。花粉濾液製備同上所述，但 AF6 緩衝液使用量為 1.8 mL，之後將花粉濾液平均分成 6 份，將每份濾液再以 AF6 緩衝液定量至 500 µL 作為單次分析樣品。將 6 樣品分別置於溫度設定為 25、30、35、40、45 及 100℃之乾浴器內處理 15 min 後，以 IFC 進行花粉活力檢測，並以 100℃處理後花粉測得之細胞散點界定為死亡細胞與活力低下細胞。IFC 之參數設定條件同上一試驗所述，每一溫度處理共進行 3 重複。

統計分析方法

試驗水稻種植採用 RCBD，試驗所得資料經 SAS Enterprise Guide 7.1 (SAS Institute Inc. 2014) 套裝統計分析軟體進行 ANOVA 變方分析。若處理間差異顯著 ($P < 0.05$)，則利用最小顯著差異性測驗 (least significant difference test; LSD) 比較各處理平均值間之差異。

結果

田區與耐熱性檢定圃之水稻花粉活力表現調查

2020 年第二期作於抽穗至穀粒成熟期間，檢定圃日均溫度變化範圍在 27–35℃間，田區溫度變化範圍在 21–28℃間 (圖 1A)。在未加熱期間 (8/3–9/20) 檢定圃與田區於 08:00–15:00 (加熱) 或 15:00–08:00 (未加熱) 時段之溫度差異皆為 4.5℃，顯示因塑膠棚設施所造成的日均溫度差異為 4.6℃；檢定圃加熱期間 (9/21–11/30) 與田區溫度差異，在加熱時段為 8.7℃，在非加熱時段為 5.2℃，與田區之日均溫度差異為 6.3℃ (表 2)。5 個水稻品種 (系) 的取穗日期介於 10/14–10/22 之間 (表 1)。以 IFC 檢測當日開放穎花之花粉活力如表 3，結果顯示取自檢定圃的花粉活力在 69.3–79.5% 間；取自田區的花粉活力在 63.1–77.7% 間，同一品種 (系) 檢定圃與田區之花粉活力相較，皆以檢定圃較田區之花粉活力稍高，但統計上均顯示無明顯差異。在稔實率方面，顯示檢定圃在 81.3–91.8% 間；田區在 82.0–92.7% 間，同一品種 (系) 在不同場域之稔實率相較，亦均無顯著差異 (表 3)。

2021 年第一期作抽穗至穀粒成熟期間的日均溫度變化，檢定圃在 31–37℃間，田區在 24–30℃間 (圖 1B)。在未進行加熱期間 (3/7–4/20) 檢定圃與田區之日均溫度差異為 4.7℃，與 2020 年第二期作之日均溫度差異 4.6℃相近；加熱期間 (4/21–6/20) 檢定圃在加熱時段之均溫達 40.4℃，與田區溫度差異達 10.0℃；在非加熱時段 (15:00–8:00) 檢定圃與田區之平均溫度差異為 4.0℃，檢定圃與田區之日均溫差為 6.3℃ (表 2)。5 個水稻品種 (系) 於 4/28–5/24 間進行採穗 (表 1)。以 IFC 檢測花粉活力與花粉濃度，結果顯示同一品種 (系) 採自不同場域之花粉濃度均顯示無明顯差異 (表 4)。花粉活力檢測結果顯示，取自檢定圃之花粉活力在 57.8–74.4% 間，取自田區在 62.9–76.4% 間，5 個品種 (系) 中僅 ‘N22’ 與 PA163 在兩場域間顯示無顯著差異，其餘 3 品種 ‘TN11’、‘TK9’ 及 PA164 皆以田區花粉活力顯著高於檢定圃。稔實率方面，檢定圃以 ‘N22’ 之 89.2% 最高，其餘 4 品種在 43.5–55.4% 間，田區組在 88.7–93.0% 間，5 個品種 (系) 中僅 ‘N22’ 在不同場域顯示無顯著差異，其餘 4 品種 (系) 皆以田區組之稔實率顯著高於檢定圃 (表 4)。

田區花粉以不同溫度處理對花粉活力之影響

2021 年第一期作取田間當日開放穎花之

表 3. 2020 年第二期作高溫處理溫室與田間 5 個水稻品種 (系) 之花粉活力與稔實率。

Table 3. Pollen viability and spikelet fertility of 5 rice cultivars (lines) planted in the field or in a heating greenhouse in the second cropping season of 2020.

	Pollen viability (%)		Spikelet fertility (%)	
Cultivar/line	Heat nursery	Field	Heat nursery	Field
'N22'	69.3 ± 7.6 a[z] B[y]	63.1 ± 6.3 a C	91.8 ± 4.4 a A	92.7 ± 1.5 a A
PA163	79.5 ± 3.5 a A	77.7 ± 3.6 a A	81.7 ± 3.9 a A	82.0 ± 6.2 a B
PA164	77.8 ± 5.7 a A	74.5 ± 6.3 a AB	81.3 ± 7.5 a A	82.9 ± 5.3 a AB
'Tainan 11'	72.9 ± 6.9 a AB	71.5 ± 6.5 a ABC	86.7 ± 6.5 a A	84.2 ± 5.4 a AB
'Taiken 9'	69.8 ± 4.3 a B	65.4 ± 11.5 a BC	86.3 ± 4.8 a A	91.9 ± 1.7 a AB

[z] Means with different letters (in small letter) in the same cultivar (lines) in the same character are significantly different ($P < 0.05$) by *t*-test.

[y] Means with different letters (in capital letter) in the same column are significantly different ($P < 0.05$) by least significant difference (LSD) test.

花粉以 25–45℃溫度處理 15 min 後，以 IFC 檢測花粉活力之變化。結果顯示 5 個水稻品種 (系) 皆以 25℃處理之花粉活力值最高，介於 61.3–65.9% 間 (表 5)。為顯示花粉活力受溫度處理影響之下降程度，將各溫度處理測得之花粉活力與 25℃處理相較，計算相對 25℃處理之花粉活力百分比 (相對花粉活力)。結果顯示在 35℃或 40℃處理後之相對花粉活力變化，以 'N22' 保持在 85.9–86.8% 間最高；下降幅度次低者為 'TN11' 與 PA164，在 40℃處理時相對活力仍保持在 60% 以上；而以 PA163 與 'TK9' 下降幅度最大，在 35℃時相對活力皆已降至 60% 以下，其中 'TK9' 隨處理溫度上升，花粉活力下降之幅度最為明顯。依照溫度處理後各品種 (系) 相對花粉活力保持程度加以排序，由高至低分別為 'N22'、'TN11'、PA164、PA163 及 'TK9'。

討論

不同期作對檢定圃高溫處理之影響

在全球氣候持續暖化的大趨勢下，長期高溫已然成為臺灣農業必須面對的新常態。水稻是臺灣最重要的糧食作物，耐熱水稻的育成有其必要與急迫性，但如何有效篩選水稻的耐熱特性則是育種實施的第一步 (Prasad *et al.* 2006; Chuang & Lur 2013)。一般而言，植物在生殖生長階段較營養生長階段對高溫更為敏感，水稻花粉受到高溫影響會導致花粉活力與花藥囊開裂比率降低，進而影響散粉、柱頭上的花粉數、受精率以及後續的穀粒發育，最終反應於米質與產量的降低 (Fahad *et al.* 2018)。本研究於農試所之田區設置改良式雙拼圓頂力霸塑膠型溫室作為耐熱性檢定圃，在其內以水田方式栽培水稻，於抽穗後至穀粒成熟期間進行高溫處理，並於穎花開放當日進行花粉活力檢測。2020 年第二期作加熱時段之設定溫度為 38.0℃，實際測得之平均溫度為 37.0℃ (表 1)。依據 Kobayashi *et al.* (2011) 研究顯示，使用 37.5℃可區分耐熱與高溫敏感水稻品種，本研究 2020 年二期作與 Kobayashi *et al.* (2011) 使用之溫度相近似，但本研究同一品種 (系) 檢定圃之花粉活力與田區相較，5 品種 (系) 皆顯示無顯著差異，稔實率亦同 (表 3)，據此推測本研究使用之 5 品種 (系) 應皆非高溫敏感水稻品種 (系)。

整體來說 2020 年二期作 5 品種 (系) 高溫檢定圃與田間相較，無論在花粉活力或稔實率方面皆顯示為差異不顯著，並未能將 5 品種 (系) 的耐熱特性加以區分。因此，2021 年第一期作將白天加熱溫度由 38℃提高至 39℃外，並增加夜間 20:00 至隔日 06:00 設定為 28℃之加熱處理，同時也將採穗時間由早上 9:00 延後至 10:00，確保採穗時水稻植冠內溫度已升至設定溫度。經此調整 2021 年第一期作檢定圃加熱時段實際測得之平均溫度為 40.4℃，較設定溫度 39℃高出 1.4℃ (表 2)，與 2020 年第二期作加熱時段之均溫 37.0℃相較高出

表 4. 2021 年第一期作高溫處理溫室與田間 5 個水稻 (系) 之花粉活力與稔實率。

Table 4. Pollen viability and spikelet fertility of 5 rice cultivars (lines) planted at in the field or in a heating greenhouse in the first cropping season of 2021.

	Pollen (cell mL^{-1})		Pollen viability (%)		Spikelet fertility (%)	
Cultivar/line	Heat nursery	field	Heat nursery	field	Heat nursery	field
‘N22’	4,694 ± 851 a[z] C[y]	4,695 ± 720 a C	57.8 ± 4.2 a C	62.9 ± 4.6 a C	89.2 ± 2.2 a A	88.7 ± 1.6 a A
PA163	7,251 ± 1,239 a A	8,144 ± 1,004 a A	74.4 ± 4.1 a A	76.4 ± 3.6 a A	43.5 ± 9.4 b B	91.4 ± 2.0 a A
PA164	5,016 ± 454 a BC	6,440 ± 1,290 a B	67.3 ± 2.6 b B	72.3 ± 1.6 a B	55.4 ± 23.7 b B	90.4 ± 7.4 a A
‘Tainan11’	6,171 ± 1,624 a AB	7,409 ± 1,910 a AB	69.3 ± 5.0 b AB	75.8 ± 1.3 a AB	53.6 ± 15.5 b B	93.0 ± 1.3 a A
‘Taiken 9’	6,370 ± 757 a AB	6,296 ± 1,222 a BC	69.5 ± 3.9 b AB	75.9 ± 1.4 a AB	43.9 ± 26.7 b B	90.0 ± 1.5 a A

[z] Means with different letters (in small letter) in the same cultivars (lines) in the same character are significantly different ($P < 0.05$) by *t*-test.

[y] Means with different letters (in capital letter) in the same column are significantly different ($P < 0.05$) by least significant difference (LSD) test.

表 5. 2021 年第一期作田間 5 個水稻品種 (系) 花粉以不同溫度處理後之花粉活力與其相對其 25℃花粉活力之花粉相對活力表現。

Table 5. Pollen viability of 5 rice cultivars (lines) treated with various temperature and its relative viability relative to those at the treatment of 25℃. Pollens were collected from the field in the first cropping season of 2021.

	‘N22’		PA163		PA164		‘TN11’		‘TK9’	
PollenTreated (℃)	Pollen viability (%)	Relative viability (%[z])	Pollen viability (%)	Relative viability (%)	Pollen viability (%)	Relative viability (%)	Pollen viability (%)	Relative viability (%)	Pollen viability (%)	Relative viability (%)
25	62.3 ± 1.4 a[y]	97.8 ± 2.2 a	67.7 ± 5.0 a	94.6 ± 7.0 a	65.9 ± 3.6 a	96.6 ± 5.3 a	61.3 ± 0.6 a	99.0 ± 0.9 a	63.9 ± 12.4 a	84.0 ± 16.3 a
30	59.9 ± 1.8 a	96.2 ± 1.7 a	52.7 ± 6.8 b	77.7 ± 5.4 b	53.1 ± 7.6 b	80.3 ± 7.3 b	54.4 ± 2.9 b	88.7 ± 4.2 b	47.4 ± 10.5 ab	74.0 ± 2.3 ab
35	54.1 ± 1.3 b	86.8 ± 1.4 b	38.2 ± 5.7 c	56.2 ± 4.5 c	45.9 ± 8.6 bc	69.4 ± 10.1 bc	47.4 ± 2.2 c	77.3 ± 3.5 c	37.4 ± 11.8 b	58.6 ± 15.6 b
40	53.5 ± 0.9 b	85.9 ± 2.7 b	37.4 ± 6.9 c	55.0 ± 6.9 c	39.8 ± 4.6 cd	60.3 ± 4.0 cd	40.2 ± 4.6 d	65.6 ± 7.2 d	20.2 ± 2.4 c	32.1 ± 3.8 c
45	46.5 ± 1.7 c	74.8 ± 4.4 c	34.6 ± 8.4 c	50.8 ± 9.5 c	33.2 ± 3.8 d	50.3 ± 3.5 d	36.3 ± 4.4 d	59.1 ± 6.8 d	13.4 ± 3.7 c	20.8 ± 3.4 c

[z] Relative viability (%) = viable % at various temperature treatment/viable % at 25℃ × 100%

[y] Means with different letters in the same column are significantly different ($P < 0.05$) by least significant difference (LSD) test.

3.4℃ (表 2)。Matsui *et al*. (2001b) 以 35.0、37.5 及 40.0℃三種溫度處理 9 個栽培種水稻連續 6 d，並調查柱頭上花粉數與稔實率的關聯性，結果顯示對高溫敏感的 'Hinohikari' 在 37.5℃處理時稔實率已低於 50%，而耐高溫的 'Akitakomachi' 需提高至 40.0℃時方有相同效果，利用 3.0℃差異即可區分出高溫敏感與耐高溫的品種。本研究 2021 年第一期作檢定圃在加熱時段之均溫達 40.4℃與 Matsui *et al*. (2001b) 使用之 40.0℃高溫情境相似，但結果只有極耐高溫之 'N22' 仍維持正常稔實率，並顯著高於其他品種 (系)，其餘 4 品種 (系) 其檢定圃之稔實率與田區相較皆差異顯著，但 4 品種 (系) 間比較則差異皆不顯著 (表 4)。據此推測，4 品種 (系) 適當之高溫篩選溫度應在 37.0℃ (2020 年第二期作加熱時段均溫) 與 40.4℃ (2021 年第一期作加熱時段均溫) 之間。Tenorio *et al*. (2013) 對國際稻米研究所 (International Rice Research Institute; IRRI) 水稻種原進行耐熱篩選，指出對於高溫敏感品種如 'IR64'，在開花期進行高溫處理只要超過 37℃，即便升高僅 1℃，稔實率也會明顯下降。因此，建議以 37–38℃進行一般水稻族群之耐熱篩選，而以 38–39℃進行極耐熱品種之篩選。在本研究中 2020 年第二期作高溫檢定圃設定溫度為 38.0℃，實際加熱時段平均溫度為 37.0℃，亦即這樣的溫度設定在二期作之環境下並未能區別出品種之耐熱性，這點可從檢定圃花粉活力較田區為高得到佐證，雖然兩者在統計上無明顯差異 (表 3)；2021 年第一期作高溫檢定圃設定溫度為 39.0℃，實際加熱時段平均溫度為 40.4℃，一期作之環境高溫讓檢定圃的實際溫度較設定溫度高出許多，如表 1 中顯示之取穗當日最高溫度，除極耐高溫之 'N22' 外，推測已超出其餘 4 品種 (系) 之高溫臨界點。

高溫對水稻的影響並非只有溫度本身，許多環境的因素亦同時與溫度交感，加重或減弱溫度的影響力。Matsui *et al*. (2014) 在澳洲新南威爾斯的水稻試驗顯示，儘管開花期的田間溫度高達 40℃，但檢視稔實率並未受到明顯影響，推測雖然氣溫很高但因當地濕度較低 (20.7%) 植冠內蒸散作用旺盛，穗溫與氣溫之差異可達 4–6.8℃，亦即在低濕度、有風的狀態下降低了高溫的危害。Matsui *et al*. (1997) 以 37.5℃配合 3 種濕度處理觀察對稔實率之影響，結果顯示隨著濕度增加，高溫對稔實率的影響加劇，而降低濕度則有助蒸散作用進行，因而達到降低穗溫的效果。本研究中使用之塑膠棚溫室在加溫過程中須具一定密閉性，推測塑膠棚內伴隨水田栽培的高濕度狀態，可能加劇高溫誘導穎花不稔的影響。綜合上述建議高溫檢定圃之溫度設定須考慮與期作環境、設施種類的交感並取得平衡點，以避免高溫處理實際效果的過與不及。

影響高溫處理效果之各種因子

Satake & Yoshida (1978) 觀察造成稔實率下降的原因，指出高溫敏感品種的障礙在於花粉活力低下造成花藥囊未能正常開裂，因影響散粉而造成柱頭花粉數的不足；而耐熱品種花藥囊雖能正常開裂，柱頭上之花粉數正常但花粉在柱頭上的萌發率降低。Matsui *et al*. (2001b) 研究指出 3 種溫度與柱頭上花粉數或稔實率的關聯性，在 37.5℃處理柱頭上花粉數大於 10 之百分比與稔實率吻合，但 40.0℃處理其柱頭上花粉數大於 10 之百分比與稔實率相關性變低，顯示極端高溫不只影響花粉散出與萌發 (柱頭上花粉數)，同時持續影響後續花粉管的伸長與受精作用，最終反應於稔實率的下降。此外，Matsui *et al*. (2021) 以多重回歸分析高溫導致穎花不稔 (heat induced flower sterility; HIFS) 的各種影響因子，結果亦顯示 HIFS 與花粉在柱頭萌發之後的過程相關性最高。本研究 2020 年第二期作檢定圃 5 品種 (系) 高溫處理期間每日之最高溫度在 39.6–42.6℃間 (表 1)；2021 年第一期作 'N22' 採穗前 1 wk 高溫處理期間每日最高溫度範圍在 37.8–41.5℃間 (資料未顯示)，其餘 4 品種 (系) 則在 41.1–45.6℃間 (表 1)。2021 年第一期作雖然高溫處理中加熱時段平均溫度為 40.4℃，但加熱時段 (亦為開花時段) 中之最高溫度則更高，推測這樣的高溫可能影響生殖或生理的層面更為深廣，在表 4 中亦可見稔實率受到高溫影響的程度較花粉活力為高。

高溫處理期程的長短亦能造成影響，Kobayashi *et al*. (2011) 研究顯示延長高溫處理

時間從 1 d 至 3 d，高溫累積效果會影響花粉膨壓進而影響花藥囊開裂，而花藥囊開裂比率又與小穗稔實率高度相關。本研究高溫處理從抽穗至穀粒成熟期，研判高溫累積效應較 Kobayashi *et al*. (2011) 之 3 d 或 Matsui *et al*. (2001b) 的 6 d 更為嚴重，高溫持續時間短時，影響包括與花粉活力高度相關的花藥囊開裂或花粉飛散能力；高溫持續時間長時，影響則包括花粉於柱頭萌發及之後的諸多生殖過程，如花粉管伸長與完成受精等功能。從實務上來看，花粉在開花時對溫度的反應敏感，但一朵花內花藥囊開裂至受精完成之時間僅約 1 h 許，而稔實率則涵蓋一個小穗上所有之花朵，因此若長時間處於高溫下，推測稔實率受到溫度累積效應之影響會較花粉活力為高。本研究檢定圃高溫處理之啟動係以最早開花的 ‘N22’ 為準，因此較晚開花品種 (系) 接受高溫處理期程相對較長，2020 年第二期作 ‘N22’ 因延遲採穗 (9/21 抽穗開始高溫處理，10/14 採穗)，因此 ‘N22’ 高溫處理的期程與其他品系相近。然而 2021 年第一期作於 4/21 開始高溫處理，‘N22’ 於 4/28 採穗，‘N22’ 高溫處理期程相較其他品系約短 3–4 wk (表 1)，推測其餘 4 品種 (系) 除實際受到較高溫度外，高溫處理期程亦較 ‘N22’ 為長，導致稔實率呈現明顯下降 (表 4)。本研究為合作計畫，另有工作目標為高溫對米質之影響，因此高溫處理從抽穗期延伸至穀粒成熟，然而花粉活力檢測最終目的用於解釋稔實率受高溫逆境的影響，因此高溫處理之期程應以含蓋小穗受精完成即可，方能正確呈現高溫處理對花粉活力與稔實率之影響以及兩者相對之關係。

耐溫性花粉活力檢測

本研究 5 品種 (系) 取自田間的花粉濃度與花粉活力本來就存在高低差異，但皆足以達成正常稔實率的要求，應視為品種基因型的特性表現 (表 3、表 4)。然而各品種花粉在經過不同溫度處理後，其花粉活力隨溫度改變之程度則呈現明顯差異，亦即同一品種的花粉活力在未達其臨界溫度前，對於溫度的反應具有一貫性 (Hsia *et al*. 2022)。本研究以取自田區水稻之花粉進行不同溫度處理後檢測其活力的變化，結果顯示 5 個品種 (系) 花粉活力皆隨處理溫度上升而下降 (表 5)，表示提高處理溫度會造成花粉活力下降，但各品種花粉活力隨著溫度上升而下降的比例並不相同，顯示不同品種花粉對溫度處理的反應並不相同。為讓不同品種水稻在同一基準下進行比較，將各品種不同溫度處理後的花粉活力與該品種在 25℃處理時之花粉活力 (最高值) 相比之百分比作為相對花粉活力，再以相對花粉活力進行溫度處理影響的比較 (表 5)。5 個品種 (系) 依相對花粉活力受溫度影響下降之幅度由小至大加以排序，依序為 ‘N22’、‘TN11’、PA164、PA163 及 ‘TK9’，此一相對花粉活力下降幅度排序與 5 個品種 (系) 在高溫檢定圃之稔實率排序相符合，亦即 ‘N22’ 最高、PA164 與 ‘TN11’ 其次、PA163 與 ‘TK9’ 最低 (表 4)。值得一提的是，本研究使用之 PA164 與 PA163 是以 ‘TK9’ 導入 ‘N22’ 耐熱基因 *qHTSF4.1* 所育成之近同源系 (BC_4F_5) 雜交水稻，觀察其花粉活力在高溫下表現皆較 ‘TK9’ 有明顯提升 (表 3、表 4)，推測與導入 ‘N22’ 耐熱基因提高了花粉的耐熱特性有關，其中 PA163 雖然在檢定圃高溫處理下有較高之花粉活力，但其稔實率表現仍然偏低 (表 4)，然而從花粉溫度處理可以看到 PA163 在 35℃處理時相對花粉活力有較明顯之下降 (表 5)，顯示利用不同溫度處理花粉與檢定圃高溫處理相較，對花粉耐熱特性的瞭解更為清晰。Matsui *et al*. (2007) 指出高溫導致的穎花不稔與品種間的關係並不單純而是非常複雜的組合關係，建議育種者可藉由各種可視性狀如開花時間、花藥特性、植株外型 (花穗角度與高度) 等進行篩選。

結語

花粉對環境因子的改變相當敏感，除受基因型影響外，還受到陽光輻射、大氣溫度及壓差 (濕度) 共同的影響 (Kobayashi *et al*. 2010)。例如同一田區中因水稻植冠所在位置的不同，或同一植冠內不同花穗或同一花穗在不同時間開放之穎花，其受到各種環境因子影響的程度並不相同。因此，有研究報告建議此類研究應該標記高溫處理下開放之穎花並追蹤

這些穎花受到的影響，方能正確追蹤高溫所造成的影響 (Jagadish *et al*. 2007)。然而回歸執行層面，若要在育種過程中進行這樣的調查，在實施上有其困難度而且相當耗費人力。Paupière *et al*. (2014) 指出在高溫下花粉活力的表現與耐熱特性具有直接相關性，花粉耐受高溫的能力直接影響花藥囊的開裂已經得到證實，且後續柱頭散粉、花粉管伸長及授精作用等生殖過程依然是花粉功能的延伸 (Zhao *et al*. 2010; Kumar *et al*. 2015)。本研究證明利用 IFC 可快速檢測花粉活力與花粉在不同溫度處理活力之改變，可作為高溫檢定圃進行前的大量種源篩選或初級篩選之用，除具節省成本提高篩選效率之優點外，亦能提供育種者或農民作為選擇之參考。未來將以目前建立之花粉溫度處理方法為基礎，進行更多花粉耐溫處理與稔實率相關之研究，期能更精確瞭解各品種花粉對不同溫度之反應，並持續改進耐高溫篩選之效率，作為吾人因應氣候變遷挑戰的依據。

誌謝

本研究承蒙行政院農業部 110 農科-10.2.1-農 -C1 計畫經費支持，謹此致謝。本研究工作承研究室同仁蘇永傑先生協助試驗，特一併表達感謝。

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Effects of High Temperature after Heading Stage on Rice Pollen Viability and Fertility

Chi-Ni Hsia[1], Charng-Pei Li[2], Wei-Ting Tsai[3], Yi-Heng Tsai[4], Pei-Ying Huang[4], Ching Chuang[5], and Shuen-Chi You[5,*]

Abstract

Hsia, C. N., C. P. Li, W. T. Tsai, Y. H. Tsai, P. Y. Huang, C. Chuang, and S. C. You. 2024. Effects of high temperature after heading stage on rice pollen viability and fertility. J. Taiwan Agric. Res. 73(4):281–291.

This study conducted heating treatment beginning from rice heading to grain maturity in a greenhouse to investigate the effects of high temperature on pollen viability and spikelet fertility. Rice materials including heat-resistant early-maturing indica upland rice 'Nagina 22' ('N22'), medium-late-maturing japonica rice 'Tainan 11' ('TN11') and 'Taiken 9' ('TK9'), as well as PA163 and PA164, the BC_4F_5 lines bred by 'TK9' with 'N22' heat-resistant gene, were planted in the second cropping season of 2020 (2020-2) and the first cropping season of 2021 (2021-1), respectively, using paddy field cultivation methods in field and plastic greenhouse. The greenhouse was heated with a diesel heater from 08:00 to 15:00 every day after 'N22' heading to grain maturity, with a set temperature of 38℃; in the 2021-1 cropping season, the set temperature during the day is 39℃ and at night heating treatment was performed from 20:00 to 06:00 the next day with a set temperature of 28℃. The plants were tagged on the heading day and the ears were picked 2 d later. The pollen from the spikelet that was about to open on the day of ear picking was used to test the pollen viability using impedance flow cytometry (IFC). The results of the 2020-2 cropping season showed that there was no significant difference in pollen viability or spikelet fertility between the field and the heated greenhouse in the same variety. The results of the 2021-1 cropping season showed that 3 of the 5 varieties had significant differences in pollen viability between the field and the heated greenhouse, namely, PA164, 'TN11' and 'TK9'. In addition to the above 3 varieties, there was another line, PA163, that showed a significant difference in spikelet fertility. Meanwhile, in the 2021-1 cropping season, the field pollen of 5 varieties (lines) was collected and treated in the range of 25–45℃, and the changes in pollen viability were tested. The pollen viability measured at various temperature treatments was compared with the pollen viability of the 25℃ treatment (the highest value) as the relative pollen viability. The degree of influence of temperature on the relative pollen viability was sorted from small to large, which were 'N22', 'TN11', PA164, PA163, and 'TK9', respectively. The results of this study showed that both pollen viability and spikelet fertility were affected by high temperatures. However, using greenhouse heating treatment was costly, with less flexibility on temperature settings compared to the treatment using pollen collected from the field with various temperatures and testing changes of its viability, which has the advantages of simplicity, ease of implementation, and high efficiency. Therefore, it is recommended that the impact of high temperature on pollen viability be used as a rapid or primary screening method for heat-resistant rice evaluation.

Key words: *Oryza sativa* L., Spikelet fertility, Pollen viability, Impedance flow cytometry.

Received: July 2, 2024; Accepted: September 23, 2024.

[*] Corresponding author, e-mail: yousc_1@tari.gov.tw

[1] Research Fellow, Crop Genetic Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

[2] Research Fellow, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

[3] Contract Associate Research Fellow, Crop Genetic Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

[4] Project Assistants, Crop Genetic Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

[5] Assistant Research Fellows, Crop Genetic Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 73(4):293 (2024)
https://doi.org/10.6156/JTAR.202412_73(4).0007

Research Article

勘誤：Gut Microbiota of 3 Beetle Larvae and Their Potential for Humic Acid Transformation

Tai-Chuan Wang[1], Chien-Yong Yeh[2], Yu-Cheng Lin[2], Bo-Wen Lin[3], Me-Chi Yao[4], and Shu-Chen Chang[4,*]

73 卷 3 期第 181 頁英文摘要倒數第 2–5 行文字有誤，應更正如下：

To further examine the ability of strains to convert rice straw into humic acid, *B. megaterium* BM01, *B. aryabhattai* BA01, and *B. subtilis* BS01 exhibited an increased humic acid conversion efficiency of 2.2–2.4%, compared to the control group without inoculation.

第 196 頁中文摘要倒數第 2–3 行文字有誤，應更正如下：

進一步檢驗菌株將稻草轉化為腐植酸的能力，其中 *B. megaterium* BM01、*B. aryabhattai* BA01 及 *B. subtilis* BS01 之腐植酸轉換效率，較未接菌種的對照組增加 2.2–2.4%。

* Corresponding author, e-mail: scchang@tari.gov.tw
1 Assistant Research Fellow, Crop Environment Section, Kaohsiung District Agricultural Research and Extension Station, Pingtung County, Taiwan, ROC.
2 Project Assistants, Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
3 Associate Technical Specialist, Taichung Harbor Inspection Station, Taichung Branch, Animal and Plant Health Inspection Agency, Taichung City, Taiwan, ROC.
4 Associate Research Fellows, Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

台 灣 農 業 研 究

(原《中華農業研究》期刊)

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台灣農業研究　評閱者名單

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石信德
農業試驗所植物病理組

石憲宗
農業試驗所應用動物組

吳文哲
國立臺灣大學昆蟲學系暨研究所

吳明城
國立中興大學昆蟲學系

呂依儒
國立臺灣大學植物病理與微生物學系

李奇峰
農業試驗所應用動物組

李雅琳
農業試驗所遺傳資源與生物技術組

邵貽沅
實踐大學食品營養與保健生技學系

姚美吉
農業試驗所應用動物組

洪滉祐
國立嘉義大學生物機電工程學系

張正
國立中興大學園藝學系

張宗仁
美國喬治亞大學植物病理學系

張哲瑋
農業試驗所嘉義分所園藝系

許正一
國立臺灣大學農業化學系

許如君
國立臺灣大學昆蟲學系

陳宗祺
亞洲大學生物科技學系

陳宗禮
國立中興大學農藝學系

陳建德
國立中興大學農藝學系

陳昶霖
國立中興大學園藝學系

陳健忠
農業試驗所應用動物組

陳榮坤
臺南區農業改良場

黃健瑞
國立嘉義大學植物醫學系

楊永裕
國立屏東科技大學植物醫學系

楊純明
農業試驗所作物組

葉信宏
中央研究院農業生物科技研究中心

蔡文錫
國立嘉義大學植物醫學系

鄭宇伸
國立雲林科技大學化學工程與材料工程系

鄭安秀
臺南區農業改良場

鄭明倫
國立自然科學博物館生物學組

蕭巧玲
農業試驗所作物組

賴永昌
農業試驗所嘉義分所農藝系

賴宏亮
國立屏東科技大學農園生產系

謝廣文
國立中興大學生物產業機電工程學系

鍾嘉綾
國立臺灣大學植物病理與微生物學系

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Volume 73, 2024

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JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol. 73 No. 4 December 31, 2024

Published by Taiwan Agricultural Research Institute, Ministry of Agriculture
Publisher Hsueh-Shih Lin
Editor-in-Chief Hsien-Tzung Shih
Address No.189, Zhongzheng Rd., Wufeng Dist., Taichung City 413008, Taiwan, ROC
Website https://www.tari.gov.tw/jtar; E-mail: jtar@tari.gov.tw; Tel: +886-4-2331-7236
Submission https://www.ipress.tw/J0042
Complete instructions to authors are available on Journal of Taiwan Agricultural Research website: https://www.tari.gov.tw/jtar/

Press Ainosco Press
Address 18F, No.80, Sec.1, Chenggong Rd., Yonghe Dist., New Taipei City 234634, Taiwan, ROC
Tel +886-2-2926-6006
E-mail press@airiti.com

Price NT$ 175 (per copy)
Distributor 1. GB Books / 1F., No.209, Songjiang Rd., Taipei City 104472, Taiwan, ROC
GB Website: http://www.govbooks.com.tw; Tel: +886-2-2518-0207
2. Wunan Books / No.6, Zhongshan Rd., Taichung City 400002, Taiwan, ROC; Tel: +886-4-2226-0330